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Parminder Chahal

*University of Nebraska-Lincoln*

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**CONTROL OF HERBICIDE-RESISTANT VOLUNTEER CORN IN  
HERBICIDE-RESISTANT SOYBEAN**

by

Parminder Chahal

A THESIS

Presented to the Faculty of  
The Graduate College at the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree of Master of Science

Major: Agronomy

Under the Supervision of Professor Amit J. Jhala

Lincoln, Nebraska

December, 2014

# CONTROL OF HERBICIDE-RESISTANT VOLUNTEER CORN IN HERBICIDE- RESISTANT SOYBEAN

Parminder Chahal, M.S.

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Adviser: Amit J. Jhala

Volunteer corn is a problem weed in soybean fields because it reduces yield and seed quality, and potentially harbors insects, pests, and diseases. Several pre-packaged herbicides have been registered in soybean in recent years, but response of volunteer corn to these herbicides has not yet been documented. Therefore, the first objective of this study was to evaluate the response of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn to 20 pre-emergence (PRE) and 17 post-emergence (POST) soybean herbicides. The results indicated that PRE soybean herbicides partially controlled ( $< 80\%$ ) volunteer corn except clomazone, while acetyl CoA carboxylase (ACCase) inhibiting herbicides provided  $\geq 85\%$  control. Germination and emergence are critical stages in weed seed establishment and persistence. Scientific literature is not available about the factors affecting germination and emergence of volunteer corn. The second objective was to determine the effects of different environmental and agronomic factors on the germination and emergence of glyphosate-resistant hybrid and volunteer corn. The results indicated that response of hybrid and volunteer corn to majority of the variables tested was similar, suggesting that volunteer corn can germinate and emerge in

a wide range of climatic conditions. Majority of growers control volunteer corn when it is visible above the soybean canopy, but this can result in early season competition with soybean. The third objective was to evaluate the impact of different densities of glyphosate-resistant volunteer corn at different control timings, and late season volunteer corn emergence on soybean yields. Late season volunteer corn emergence had no significant effect on soybean yield. Yield did not decrease with all volunteer corn densities, except with the highest density (10,000 plants and 500 clumps ha<sup>-1</sup>) at all control timings. Soybean growers are looking for alternative herbicides, such as glufosinate, for management of glyphosate-resistant weeds, including volunteer corn. The fourth objective was to evaluate different herbicide programs for control of glyphosate-resistant volunteer corn in glufosinate-resistant soybean. The results suggested that glufosinate applied at different rates in a single or sequential application provided  $\geq 85\%$  control of volunteer corn along with other weeds. These results will provide useful information to soybean growers for management of volunteer corn.

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## Chapter 1

### Literature Review

#### Corn and Soybean Production

Corn (*Zea mays* L.) is an annual, monoecious plant having male and female reproductive parts on the same plant (Kiesselbach, 1999). The United States is the largest producer of corn in the world (USDA, 2013). In 2013, the estimated area planted to corn in the United States was about 35.39 million ha (USDA, 2013). This number is expected to increase to 38 million ha by 2016 (Malcolm & Aillery, 2009). Nebraska is the third largest producer of corn in the United States with the planting area of 3.8 to 4 million ha annually. Corn is commonly used as human food, fuel production, livestock feed, and sold as an export commodity (Farnham, et al., 2003; Windham and Edwards, 1999). In 2013, corn varieties resistant to herbicides, insects, or a combination of both the traits occupied 91% of total corn area (USDA-NASS 2013). Increased cultivation of herbicide-resistant corn has raised concerns about herbicide-resistant volunteer corn during soybean season in corn-soybean rotation (Marquardt et al., 2013). Soybean (*Glycine max* L.) is native to eastern Asia (Hymowitz, 1990) and was first introduced in the United States in 1765 (Hymowitz and Harlan, 1983). Soybean is ranked as one of the most important crops worldwide, primarily grown as an oil seed crop for livestock feed and biofuel feedstock and the United States is the largest soybean producer in the world (Masuda and Goldsmith, 2009). Glyphosate-resistant corn and soybean were commercialized in the

late 1990s, and since then, have been adopted rapidly by growers, primarily in the Americas.

### **Volunteer Corn**

Volunteer corn results from the overwintering of the hybrid corn used the previous year or from a failed corn stand in replanted corn (Steckel et al., 2009; Shauck & Smeda, 2012). Storm damage, harvesting problems, poor stalk quality, and insect damage, among other factors, can lead to kernel and ear losses that result in volunteer corn the following year. Volunteer corn was documented as a weed even before the commercialization of glyphosate-resistant corn (Andersen et al., 1982; Beckett & Stroller, 1988), with glyphosate used in rope-wick applications to control volunteer corn (Andersen et al., 1982; Beckett & Stroller, 1988; Dale, 1981). No-till management system is gaining popularity as growers can still maintain profitable crop production while reducing labor and fuel inputs (Brown et al., 1989; Griffith et al., 1986; Hairston et al., 1984); however, weed control under no-till normally depends on the use of herbicides in modern agriculture (Buhler, 1988; Coffman and Frank, 1991; Koskinen and McWhorter, 1986). The adoption of no-till corn-soybean systems has favored survival of volunteer corn as corn seeds are left on the surface or in shallow soil depths unlike under conventional tillage system where seeds buried to deeper depths (Steckel et al., 2009).

### **Impact of Volunteer corn on the Soybean Yield**

Volunteer corn is a competitive weed and can reduce soybean yield through competition during the growing season. Previous studies found that volunteer corn reduced yield in crops grown in rotation, including corn (Jeschke and Doerge, 2008),

cotton (*Gossypium hirsutum* L.) (Clewis et al., 2008), soybean (Beckett and Stroller, 1988), and sugarbeet (*Beta vulgaris* L.) (Kniss et al., 2012). Jeschke and Doerge (2008) reported a 1.5 to 13% corn grain yield loss at a volunteer corn density of 0.5 to 4 plants  $\text{m}^{-2}$ . Clewis et al. (2008) reported 4 to 8% cotton lint yield loss with each 500 g increase in volunteer corn biomass per meter of the crop row. Kniss et al. (2012) reported 19% sucrose yield loss in sugarbeet at volunteer corn density of 1 to 1.7 plants  $\text{m}^{-2}$ . A uniform corn density of 0.4 plants  $\text{m}^{-1}$  of soybean row caused a 14 to 49% yield reduction depending on the location and year (Andersen et al., 1982). Wilson et al. (2010) reported that volunteer corn density of 8,750 and 17,500 plants  $\text{ha}^{-1}$  reduced soybean yields by 10 and 27%, respectively, in Nebraska. Clumps of volunteer corn plants cause more soybean yield loss compared to individual plants. Andersen et al. (1982) reported reduction in soybean yield from 31 to 83% with increase in volunteer corn clump density from 1 to 4 clumps spaced every 2.4 m of soybean row.

### **Volunteer Corn and Western Corn Rootworm**

*Bacillus thuringiensis* corn hybrids (GM plants) produce insecticidal toxins in their tissues and resist feeding by specific insect pests. These hybrids are increasingly being stacked with other transgenic traits such as glyphosate and glufosinate. Western corn rootworm, *Diabrotica virgifera virgifera*, is one of the most devastating corn insect pests in the United States (Levine and Oloumi-Sadeghi, 1991; Sappington et al., 2006). It overwinters in the egg stage in the soil and eggs are deposited in the soil during the summer. Rootworm larvae can complete development only on corn and a few other species of grasses. Larvae feeds on corn roots before pupating out of the soil. Feeding on

corn roots can cause root injury and reduce corn growth and yield (Godfrey et al., 1993; Gray and Steffey, 1998). Adults feed primarily on corn silk, pollen and kernels on exposed ear tips, although they also feed on leaves and pollen of other plants. Volunteer corn present in the soybean field provides feeding option to the corn rootworms and, thus, it limits the benefits of corn-soybean rotation and creates challenges for insect-resistance management (Marquardt et al., 2012; Krupke et al., 2009; Shaw et al., 1978).

### **Volunteer Corn Management**

The acetyl-coenzyme A carboxylase (ACCase) inhibiting-herbicides, also known as graminicides, are often used in soybean to control grass weeds, including volunteer corn. Several studies reported that diclofop, fluazifop, quizalofop, and sethoxydim were effective for controlling volunteer corn in soybean (Andersen, 1976; Andersen et al., 1982; Andersen & Geadelmann, 1982; Beckett & Stroller, 1988; Beckett et al., 1992). Management of volunteer corn is challenging due to the fact that PRE, soil applied herbicides registered in soybean are not very effective (Beckett and Stoller, 1988). Therefore, only option to control volunteer corn in soybean is POST application of acetyl-coenzyme A carboxylase (ACCase) inhibiting-herbicides (Beckett and Stoller, 1988; Beckett et al., 1992; Deen et al., 2006; Marquardt and Johnson, 2013; Young and Hart, 1997). Majority of growers control volunteer corn when it is visible above the soybean canopy, but that results in early season competition with soybean. Soybean yield could be improved by identifying the critical period for controlling volunteer corn emerging early and late in the season. Critical period of weed control in soybean is longer under no-till system starting from VC (unrolled unifoliate leaves) or V1 (1<sup>st</sup> trifoliate) to

R1 or beginning flowering stage (Halford et al., 2001), compared to conventional tillage system (VC to V4) at 2.5% yield loss (Van Acker et al., 1993). Volunteer corn plants emerging late could provide competition to soybean and reduce yield.

### **Germination and Emergence of Volunteer Corn**

Several environmental factors affect germination and seedling emergence (Baskin and Baskin, 1998). Temperature, to which seeds are exposed, is one of the leading factors (Tozzi et al., 2014). The optimum temperature, light, pH, and seed burial depth for germination and emergence vary with the weed species (Egley and Duke, 1985). Idikut (2013) reported 41 and 31% germination of hybrid corn at 17 and 30 °C temperatures, and 24 and 12 h photoperiod, respectively. Fausey and McDonald (1985) reported reduction in corn seedling emergence after 2 d of flooding. Khayatnezhad and Gholamin (2011) reported reduction in germination of five corn cultivars with increasing salt stress levels (0 to 250 mM). Khodarahmpur (2011) observed reduction in germination of seven corn hybrids with increasing osmotic stress level. Volunteer corn exposed to various environmental and agronomic conditions may respond differently under a range of environmental factors required for germination and emergence.

### **Glufosinate-Resistant Soybean**

Glufosinate-resistant soybean was commercialized in 2009 (Craigmyle et al., 2013) providing flexibility of in-crop application of glufosinate applied once or in a sequential application depending on weed density and size (Beyers et al., 2002). Several studies reported excellent weed control in glufosinate-resistant soybean with POST-applied glufosinate (Beyers et al., 2002; Norsworthy et al., 2010; Wiesbrook et al., 2001).

However, glufosinate-resistant soybean has not been widely adopted by soybean growers in Nebraska (I. Schleufer, personal communication). This scenario may change in the future due to the evolution of glyphosate-resistant weeds and limited effective POST herbicide options in soybeans. A recent survey reported that cultivation of glufosinate-resistant soybean is increasing in the midsouthern United States, specifically for control of glyphosate-resistant Palmer amaranth [*Amaranthus palmeri* S. Wats.] (Barnett et al., 2013; Aulakh et al., 2013). It is likely that cultivation of glufosinate-resistant soybean may increase in the near future in the Midwest for control of glyphosate-resistant weeds (Kaur et al., 2014).

### **Glufosinate**

Glufosinate is a nonselective, contact, POST herbicide that inhibits the synthesis of glutamine synthetase enzyme (Wendler et al., 1990; Wild and Wendler, 1991) and results in the accumulation of ammonia within the cell up to toxic level, causing photosynthesis cessation, disruption of chloroplast structure, and vesiculation of stroma (Devine et al., 1993; Hinchey et al., 1993). Glufosinate-resistant corn and soybean provided growers an opportunity to apply glufosinate POST for controlling many troublesome weeds. Glufosinate is a broad-spectrum herbicide and the label lists 105 broadleaf and 37 grass weeds being controlled if applied at recommended rate and weed growth stage (Anonymous, 2014). Glufosinate is usually more effective on annual broadleaf weeds compared to grasses (Corbett et al., 2004; Culpepper et al., 2000; Steckel et al., 1997). For example, Culpepper et al. (2000) reported greater control (> 80%) of common lambsquarters (*Chenopodium album* L.) and prickly sida (*Sida spinosa*



L.) with single application of glufosinate compared to broadleaf signalgrass [*Urochloa platyphylla* (Nash) R.D. Webster], goosegrass [*Eleusine indica* (L.) Gaertn], and johnsongrass [*Sorghum halepense* (L.) Pers] (< 75%). Glufosinate label recommends effective control of volunteer corn when they are 25- to 30-cm tall (Anonymous, 2014); however, variable control is reported. Steckel et al. (2009) reported variability in glufosinate efficacy with height of volunteer corn plants. In contrast, Terry et al. (2012) reported no difference in control of glyphosate-resistant corn hybrids and their progenies with glufosinate. However, few studies reported that when tank-mixed with ACCase-inhibitors, glufosinate antagonized control of some annual and perennial grasses (Burke et al., 2005; Gardner et al., 2006).

## Research Summary and Objectives

Volunteer corn is overwintering F<sub>2</sub> generation of corn hybrid grown in the previous year or corn hybrid emerging from a failed corn stand in replanted corn. It is a competitive weed that can reduce yield of the crop grown in rotation. The ACCase (acetyl-coenzyme A carboxylase) inhibiting-herbicides, also known as graminicides, are the most commonly used POST herbicides in soybean to control grass weeds, including glyphosate-resistant volunteer corn. In the United States, fifteen weed species have become resistant to ACCase-inhibitors (Heap, 2014). Their continuous use for volunteer corn control may lead to resistance in other weed species. Including PRE followed by POST application is a better option for a weed management rather than using only POST herbicides. Several PRE herbicides exist for residual grass weed control in soybean; however, none of them list volunteer corn on their labels. There is a need to identify POST soybean herbicides with modes of action different from ACCase-inhibitors and to identify a PRE herbicide registered in soybean for residual control of volunteer corn.

Controlling volunteer corn in the early growth stages might be a better option from insect resistance and individual herbicide efficacy point of view. So, there is a need to identify the growth stage of volunteer corn for better control with different herbicides. The control of volunteer corn in the early stage of growth could result in soybean yield loss due to competition from late-season emergence of volunteer corn. There is a need to find out the impact of late season emerging volunteer corn plants after being controlled at different growth stages or timings. Not only limited to chemical control, integrated weed management including the use of chemical, mechanical, and cultural practices should be

followed for better weed control and to manage herbicide resistant weeds. Information on the effect of different environmental and agronomic factors on the germination and emergence of volunteer corn could aid integrated management strategies. Due to increased issues of glyphosate resistant weeds, there is also a need for alternate herbicide-resistant crops such as glufosinate-resistant soybeans for the control of existing glyphosate-resistant weeds. Glufosinate can be used to control glyphosate-resistant volunteer corn and it could also reduce the continuous use of ACCase-inhibitors. There is a need to find out the efficiency of glufosinate applied at different rates as single or sequential application.

The efficacy of an ACCase inhibitors can be affected by a number of factors, including the growth stage of the volunteer corn, the environmental conditions at the time of application, and the efficacy of the individual herbicide. Information is not available, to our knowledge, in literature about the response of volunteer corn to PRE soybean herbicides. Therefore, the first objective was to evaluate the efficacy of PRE soybean herbicides for control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn, and evaluate the efficacy of POST soybean herbicides registered for grass weed control applied at two growth stages (2-to 3- or 4-to 5-leaf stage) for control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn. We hypothesized that 1) ACCase-inhibitors applied to the 2- to 3-leaf stage volunteer corn plants would provide better control compared to the 5- to 6-leaf stage treated plants, 2) tank-mixed application of herbicides would provide volunteer corn control comparable to ACCase-inhibitors, and 3) from all the PRE herbicide tested in this study, few could provide

optimum control of volunteer corn. The results of this study will help growers to use effective PRE and POST soybean herbicides with more than one mode of action for the control of volunteer corn.

Several environmental and agronomic factors affect germination and seedling emergence. Volunteer corn exposed to various environmental and agronomic conditions may respond differently under a range of environmental factors required for germination and emergence. Literature is limited on the effect of environmental and agronomic factors on the germination and emergence of volunteer corn. The second objective was to evaluate the germination of glyphosate-resistant hybrid and volunteer maize in response to temperature, light, osmotic stress, salt stress, and pH; and the effect of seed burial depth and flooding duration on the emergence of hybrid and volunteer corn. We hypothesized that volunteer corn response to the environmental and agronomic factors in terms of germination and emergence would be different from hybrid corn tested.

Effect of different volunteer corn densities on soybean yield has been discussed in the literature. However, literature is scanty about integrated effect of volunteer corn densities, control timings, and late season emergence of volunteer corn on yield of soybean. There is a need to identify control timing of volunteer corn, present at different densities, to nullify the effect of late season emerging volunteer corn on soybean yields. The third objective was to determine the impact of different densities of volunteer corn present as individual plant or clump at different control timings, and late season volunteer corn emergence after being controlled at different soybean growth stages on soybean yields. We hypothesized that 1) the late season emergence of volunteer corn would have

an impact on soybean yield and 2) higher densities of volunteer corn controlled at later growth stages would result in soybean yield reduction.

Soybean growers are looking for alternative herbicides, such as glufosinate, for management of glyphosate-resistant weeds, including volunteer corn. It is likely that cultivation of glufosinate-resistant soybean may increase in the near future in the Midwest for control of glyphosate-resistant weeds and volunteer corn. Scientific literature is not available regarding the efficacy of glufosinate applied alone at different rates or when tank-mixed with ACCase-inhibitors for control of volunteer corn in glufosinate-resistant soybean. Hence, fourth objective was to compare efficacy of glufosinate applied at different rates in a single or sequential application for control of glyphosate-resistant volunteer corn, compare efficacy of ACCase-inhibitors applied alone or tank-mixed with glufosinate in an early-POST followed by a late-POST application of glufosinate for control of glyphosate-resistant volunteer corn and other weeds, and evaluate yield of glufosinate-resistant soybean. We hypothesized that 1) sequential application of glufosinate would result in better volunteer corn control compared to single application of glufosinate and 2) tank-mixed application of glufosinate and ACCase-inhibitors would provide better volunteer corn control compared to ACCase applied alone.

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## Chapter 2

### **Efficacy of Pre-emergence and Post-emergence Soybean Herbicides for Control of Glufosinate-, Glyphosate-, and Imidazolinone-Resistant Volunteer Corn**

#### **Abstract**

Glyphosate-resistant corn and soybean are grown in rotations in the Midwest, including Nebraska. Volunteer corn is a problematic weed in soybean fields because it causes harvest problems, reduces yield and seed quality, and potentially harbors insects, pests, and diseases. Several pre-packaged herbicides have been registered in soybean in recent years, but response of volunteer corn to these herbicides has not yet been documented. Greenhouse experiments were conducted to evaluate the response of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn to 20 pre-emergence (PRE) and 17 post-emergence (POST) soybean herbicides. Cumulative emergence of volunteer corn was not affected by PRE soybean herbicides compared with the nontreated control regardless of herbicide-resistant trait at 21 days after treatment (DAT). Although comparable with several other treatments, clomazone provided  $\geq 90\%$  control of glufosinate- and imidazolinone-resistant volunteer corn at 21 DAT. The POST soybean herbicides were applied when volunteer corn plants were at the 2 to 3 or 5 to 6 leaf stage. The ACCase-inhibiting herbicides, including clethodim, fenoxaprop plus fluazifop, fluazifop, quizalofop, and sethoxydim, provided  $\geq 96$  and  $\geq 85\%$  control of the 2 to 3 or 5 to 6 leaf stage volunteer corn, respectively, regardless of the herbicide-resistance trait at 28 DAT. Glyphosate tank mixed with acifluorfen, chlorimuron-ethyl, or

imidazolinones usually provided  $> 83\%$  control of glufosinate-and imidazolinone-resistant volunteer corn when sprayed at the 2 to 3 leaf stage at 28 DAT, but control was  $\leq 71\%$  for the 5 to 6 leaf stage volunteer corn. Similar results were usually reflected in volunteer corn biomass. It is concluded that PRE soybean herbicides partially controlled volunteer corn; therefore, ACCase inhibiting herbicides are the only highly effective option for soybean growers.

**Nomenclature:** Acifluorfen; alachlor; chlorimuron-ethyl; clethodim; clomazone; cloransulam; fenoxaprop; fluazifop; flumioxazin; fluthiacet-ethyl; fomesafen; glyphosate; glufosinate; imazamox; imazaquin; imazethapyr; indaziflam; metribuzin; pendimethalin; quizalofop; sethoxydim; s-metolachlor; sulfentrazone; thifensulfuron; trifluralin; soybean, *Glycine max* (L.) Merr.; volunteer corn, *Zea mays* L.

**Keywords:** herbicide efficacy, pre-packaged herbicides, volunteer corn biomass, volunteer corn leaf stage

## Introduction

Corn-soybean is the most prominent crop rotation in the Corn Belt in the U.S. Glyphosate-resistant volunteer corn is a problem weed not only in soybean, but also in continuous corn rotations (Marquardt et al., 2012a). With the commercialization of glyphosate-resistant corn and soybean in the late 1990s, growers rapidly adopted them in the Americas (Castle et al., 2006). In 2010, more than 70% of corn and 93% of soybean planted were herbicide-resistant, primarily glyphosate-resistant (USDA-NASS, 2010). Increased adoption of glyphosate-resistant corn resulted in increasing issues of volunteer corn. Volunteer corn also plays a role in the survival and dispersal of corn rootworm and grey leaf spot disease; therefore, it limits the benefits of corn-soybean rotation and creates challenges for insect-resistance management (Marquardt et al., 2012b; Krupke et al., 2009; Shaw et al., 1978). Volunteer corn is a competitive weed, as it grows taller than soybean, and like many other weeds, causes yield reduction by competing for light, space, nutrients, and moisture (Beckett & Stoller, 1988; Marquardt et al., 2012b).

The acetyl-coenzyme A carboxylase (ACCase) inhibiting-herbicides are often used in soybean to control grass weeds, including volunteer corn; however, the efficacy of an ACCase inhibitors can be affected by a number of factors, including the growth stage of the volunteer corn, the environmental conditions at the time of application, and the efficacy of the individual herbicide (Wilson et al., 2010). Several pre-packaged herbicide tank-mixtures have been registered in recent years and are widely used by soybean growers specifically for the control of glyphosate- and ALS inhibitor-resistant weeds.



Several PRE herbicides exist for residual grass weed control in soybean; however, none of them list volunteer corn on their labels. Information is not available, to our knowledge, in scientific literature about the response of volunteer corn to PRE soybean herbicides. In addition, several new pre-packaged herbicide tank-mixtures, such as sulfentrazone plus chloransulam-methyl (Authority™ First), sulfentrazone plus metribuzin (Authority™ MTZ), etc., have been registered for PRE weed control in soybean. These new residual herbicides may expand the weed control spectrum, though the response of herbicide-resistant volunteer corn to these herbicides is unknown. Therefore, the objectives of study were to (1) evaluate the efficacy of PRE soybean herbicides for control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn, and (2) evaluate the efficacy of POST soybean herbicides registered for grass weed control applied at two growth stages (2-to 3- or 4-to 5-leaf stage) for control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn.

## **Materials and Methods**

Greenhouse studies were conducted at the University of Nebraska-Lincoln in 2013. All PRE- and POST-applied soybean herbicides registered for grass weed control were evaluated for the control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn. The herbicide application rates were selected based on the recommended labeled rates. The hybrids of glufosinate-, glyphosate-, and imidazolinone-resistant corn were planted in 2012 at the South Central Agriculture Laboratory, University of Nebraska-Lincoln near Clay Center, Nebraska. Seeds were harvested in October 2012 and kept at room temperature until they were used for this study. A preliminary study was conducted to determine the germination percentage of volunteer corn seeds. The results suggested  $\geq 98\%$  germination for each herbicide-resistant trait (data not shown).

### **PRE Herbicide Study**

The soil used in this study was collected from a field near Lincoln, Nebraska (24% sand, 25% clay, 51% silt, and 2.7% organic matter) with known history of no herbicide usage for at least the last eight years. Ten seeds each of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn were planted at 2- to 3-cm depth in plastic pots (15 cm diameter and 15 cm height) filled with the soil. The pots were watered at field capacity. Herbicides were applied on the soil surface 1 d after planting the seeds using a chamber track bench sprayer fitted with a 8001-E nozzle (Teejet Technologies, Wheaton, IL). The experiment was laid out in a 20 x 3 factorial randomized complete block design with four replications. The two factors were 20 herbicide treatments (including nontreated control) and 3 herbicide-resistant volunteer corn traits (glufosinate-, glyphosate-, and imidazolinone-

resistant). The day/night temperature and photoperiod of the greenhouse were 28/24 °C and 14 h, respectively, and the pots were watered as required. The PRE soybean herbicides used in this study are listed in Table 2.1. Herbicide rates were selected based on the recommended labeled rates for soybean.

A cumulative number of emergences of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn were recorded at 7, 14, and 21 d after treatment (DAT). Visual estimates of control of emerged volunteer corn plants were recorded at 7, 14, and 21 d after treatment (DAT) based on a 0 to 100% scale, with 0% meaning no injury or control (healthy plant) and 100% meaning complete control or severe injury with no chance of plant survival. Volunteer corn plants were harvested at the base of the plant at 21 DAT and the fresh weight was recorded. The plants were kept in a paper bag, oven dried at 60 °C for 96 h, and dry biomass weight was recorded. The experiment was repeated again for the consistency of results.

### **POST Herbicide Study**

Three seeds each of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn were seeded at a depth of 2 to 3 cm in separate plastic pots (15 cm diameter and 15 cm height), filled with 75% commercial potting mix (Berger BM1 potting mix, Berger Peat Moss Ltd., Quebec, Canada) and 25% soil. Plants were thinned to two plants per pot at 7 days after emergence. The experiment was laid out in a 2 x 18 x 3 factorial randomized complete block design with four replications. The three factors included two heights of volunteer corn [2- to 3-leaf stage (12 to 15 cm tall) and 5- to 6-leaf stage (30 to 33 cm tall)], 18 herbicide treatments (including a nontreated control),

and three herbicide-resistant volunteer corn traits (glufosinate-, glyphosate-, and imidazolinone-resistant). Plants were watered every other day and were supplied with nutrients using fertilizer solution (Scotts Miracle-Gro Products, Inc. Marysville, OH) before 5 d of herbicide treatment. Herbicide treatments were applied when volunteer corn plants were at the 2- to 3-leaf stage (12- to 15-cm tall) or the 5- to 6-leaf stage (30- to 33-cm tall). Details of POST soybean herbicides used in this study are provided in Table 2.2. Herbicide rates used were based on recommended labeled rates for soybean. Recommended adjuvants were added to the herbicide solutions (Table 2.2). Treatments were applied using the same chamber track bench sprayer noted in the PRE herbicide study.

Visual estimates of control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn were recorded at 7, 14, 21, and 28 DAT based on a 0 to 100% scale as explained in the PRE herbicide study. Volunteer corn plants were harvested at the base of the plant at 28 DAT and the fresh weight was recorded. The plants were kept in paper bags, oven dried at 60 °C for 96 h and biomass weight was recorded. The experiment was repeated again for the consistency of results.

Data from PRE and POST soybean herbicide studies were subjected to ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc, Cary, NC). Before analysis, data were tested for normality with the use of PROC UNIVARIATE. Visual estimates of volunteer control, volunteer corn emergence, and biomass data were arcsine square-root transformed before analysis; however, back-transformed data are presented with mean separation based on transformed data. For PRE herbicide study,

herbicide treatments and corn types were the fixed effects, while replications and experimental repeats (nested within replication) were considered random effects. For POST herbicide study, herbicide treatments, volunteer corn type, and plant heights were the fixed effects, while replications and experimental repeats (nested within replication) were considered random effects. Where the ANOVA indicated treatment effects were significant, means were separated at  $P \leq 0.05$  with Tukey-Kramer's pairwise comparison test.

## Results and Discussion

### PRE Herbicide Study

The two-way interaction of herbicide treatments and volunteer corn type was significant; therefore, data are presented separately. Control of volunteer corn varied among herbicide treatments at 7 d after treatment (DAT) (Table 2.3). Control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn was in the range of 9 to 69%, 6 to 58%, and 25 to 69%, respectively, at 7 DAT. However, control was improved in a few herbicide treatments at 21 DAT. For example, although comparable with several other treatments, clomazone provided  $\geq 90\%$  control of glufosinate- and imidazolinone-resistant volunteer corn at 21 DAT. Surprisingly, clomazone was not very effective ( $< 50\%$  control) on glyphosate-resistant volunteer corn. Cumulative emergence of volunteer corn at 21 DAT was comparable with the nontreated control without difference among herbicide treatments, indicating the failure of PRE soybean herbicides to prevent volunteer corn emergence.

Sulfentrazone tank mixes usually resulted in 47 to 75% control of volunteer corn and was comparable with few other treatments, including clomazone at 21 DAT (Table 2.3). Volunteer corn biomass reflected similar results with several treatments comparable with the nontreated control that indicated control failure of PRE soybean herbicides. The overall results of the PRE soybean herbicides suggest that with the exception of clomazone for glufosinate- and imidazolinone-resistant volunteer corn, no other herbicide provided economically acceptable control. Based on these greenhouse studies, it is

concluded that PRE herbicide is not available for acceptable control of glyphosate-resistant volunteer corn in soybean.

### **POST Herbicide Study**

The three-way interaction of herbicide treatments, volunteer corn type (glufosinate-, glyphosate-, and imidazolinone-resistant), and volunteer corn height was significant. Control of volunteer corn was affected by growth stage and POST soybean herbicides (Table 2.4). The ACCase-inhibiting herbicides, including clethodim, fenoxaprop plus fluazifop, fluazifop, quizalofop, and sethoxydim, resulted in 48 to 75% control of glufosinate- and glyphosate-resistant volunteer corn at 7 DAT when sprayed at the 2- to 3-leaf stage, and usually were comparable with glyphosate tank-mix treatments. The ACCase inhibitors resulted in 28 to 45% control of imidazolinone-resistant volunteer corn at 7 DAT; however, control was improved at 28 DAT and resulted in  $\geq 96\%$  control, regardless of the resistant trait. Similarly, several studies have reported  $> 90\%$  control of volunteer corn with ACCase (Andersen, 1976; Andersen et al., 1982; Andersen & Geadelmann, 1982; Beckett & Stroller, 1988; Beckett et al., 1992; Marquardt & Johnson, 2013).

Glyphosate tank mixed with acifluorfen, chlorimuron, imazamox, imazaquin, or imazethapyr usually provided 83 to 91% and 87 to 98% control of glufosinate- and imidazolinone-resistant volunteer corn, respectively, and was comparable with an ACCase-inhibitor at 28 DAT. Acifluorfen, fluthiacet-ethyl, imazamox, imazethapyr, and imazethapyr plus acifluorfen resulted in poor control ( $\leq 57\%$ ) of volunteer corn. Results of volunteer corn control were reflected in biomass. For example, the lowest biomass ( $\leq$

1.2 g pot<sup>-1</sup>) was recorded with ACCase-inhibitor herbicides and was comparable with glyphosate tank-mix treatments. Fluthiacet-ethyl, imazethapyr, or acifluorfen resulted in the highest biomass that was comparable with the nontreated control and confirmed poor control of volunteer corn in soybean.

The POST soybean herbicides applied at the 5- to 6-leaf stage of volunteer corn resulted in variable response compared with the 2- to 3-leaf stage (Tables 2.4 and 2.5). Similarly, Marquardt and Johnson (2013) reported that clethodim applied to  $\leq 30$  cm-tall volunteer corn provided higher and more consistent control compared to 90 cm-tall plants at 14 DAT at all volunteer corn densities. All herbicide treatments resulted in  $< 40\%$  control of volunteer corn at 7 DAT. However, ACCase inhibitors resulted in 85 to 97% control at 28 DAT. Similarly, several studies demonstrated effective control of volunteer corn with ACCase inhibitors. For example, Andersen et al. (1982) reported  $> 90\%$  control of volunteer corn with diclofop. Young and Hart (1997) reported  $> 90\%$  control with sethoxydim or quizalofop. Deen et al. (2006) reported that use of a recommended adjuvant significantly improved the effectiveness of ACCase inhibitors, specifically when reduced rates were applied. Glyphosate tank mixed with acifluorfen, chlorimuron, fomesafen, imazamox, imazaquin, and imazethapyr resulted in  $\leq 71\%$  control of volunteer corn, regardless of resistant trait. The lowest volunteer corn biomass was usually recorded with ACCase inhibitors confirming results of visual control estimates at 28 DAT.

Results of the PRE soybean herbicide study revealed that clomazone resulted in  $> 90\%$  control of glufosinate- and imidazolinone-resistant volunteer corn, but  $< 50\%$



control of glyphosate-resistant volunteer corn. A predominant number of corn hybrids planted in the Midwestern United States are glyphosate-resistant, and the occurrence of glyphosate-resistant volunteer corn is more widely distributed compared to glufosinate- and imidazolinone-resistant volunteer corn. In this study, PRE or POST application of imidazolinones resulted in poor control of volunteer corn. In contrast, Young and Hart (1997) reported 70 and 83% control of volunteer corn with imazaquin and imazethapyr plus imazaquin in soybean. More research is required to identify a PRE herbicide with excellent efficacy for volunteer corn control, soybean selectivity as well as to better understand the natural range in tolerance of volunteer corn lines to herbicides.

Overall results suggest that volunteer corn can be effectively controlled with ACCase inhibitors regardless of herbicide-resistant trait. The ACCase-inhibiting herbicides were more effective and consistent ( $\geq 96\%$  control) when applied to 2- to 3-leaf stage volunteer corn compared with the 5- to 6-leaf stage ( $\geq 85\%$  control). Therefore, it is advisable to control volunteer corn with ACCase inhibitors when they are at the 2- to 3-leaf stage to avoid competition with soybean during the early growth stage. In addition, early season control is recommended from an insect resistance management standpoint, if volunteer corn plants also express transgenic Bt traits (Krupke et al., 2009). Repeated application of ACCase inhibitors for the last several years has resulted in the evolution of 44 grass weed species resistant to this herbicide chemistry (Heap, 2014). In fact, resistance to ACCase inhibitors has become the third most frequent type of weed resistance (Kukorelli et al., 2013). Therefore, in the fields with ACCase inhibiting herbicide-resistant weed(s), ACCase inhibitors should be tank-mixed with other

herbicides that can effectively control resistant weeds without antagonism. Therefore, growers should adopt an integrated volunteer corn management program that may include tillage, crop rotation, and improved cultural agronomic practices to maximize control and reduce the potential for evolution of herbicide-resistant weeds.

### **Limitation of Research Project**

In the PRE herbicide study, more than three herbicide-resistant volunteer corn traits could have been included to find out their response to different PRE herbicides.

### **Future Directions**

The results from PRE herbicide study suggested that clomazone provided  $\geq 90\%$  control of glufosinate- and imidazolinone-resistant volunteer corn and  $< 50\%$  control of glyphosate-resistant volunteer corn. This difference in control could be due to natural tolerance of volunteer corn variety to clomazone, not due to the glyphosate-resistant trait. In future, clomazone could be tested on all the corn varieties that are commonly planted by growers in the United States. The response of volunteer corn or their hybrids to clomazone could help provide residual control to volunteer corn.

## Figures

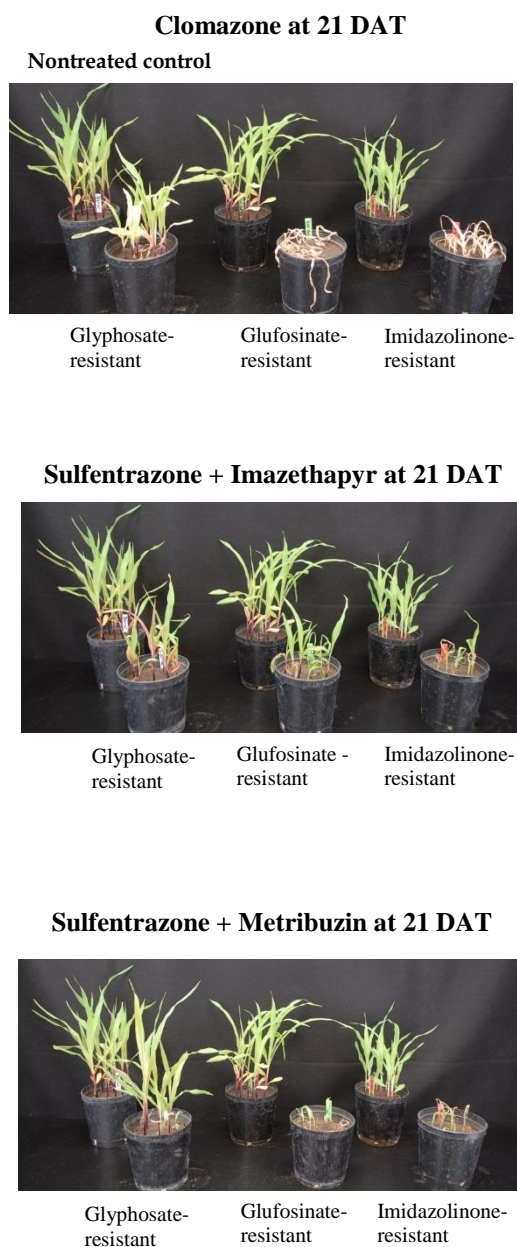


Figure 2.1. Effect of PRE herbicides on glyphosate-, glufosinate- and imidazolinone-resistant volunteer corn at 21 DAT. Nontreated controls are present in the back row for comparison.

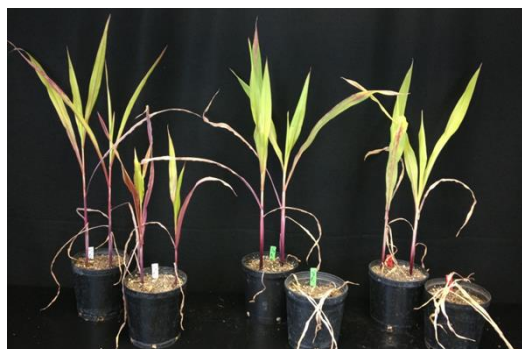
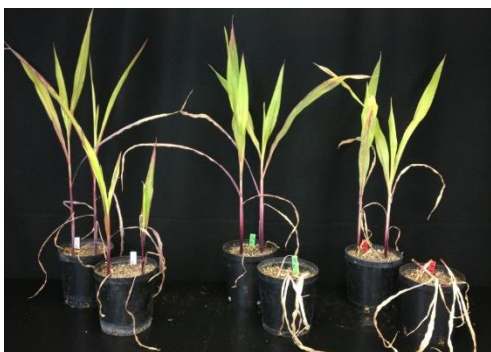
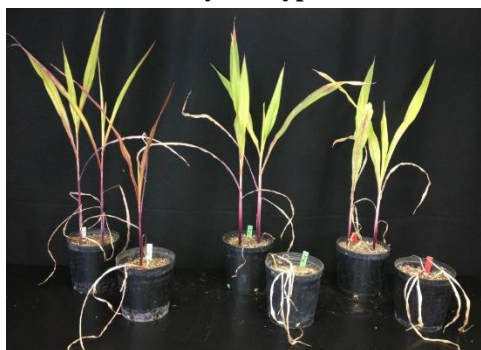
**Imazethapyr + Glyphosate at 28 DAT**Glyphosate-  
resistantGlufosinate-  
resistantImidazolinone-  
resistant**Imazaquin + Glyphosate at 28 DAT**Glyphosate-  
resistantGlufosinate-  
resistantImidazolinone-  
resistant**Chlorimuron ethyl + Glyphosate at 28 DAT**Glyphosate-  
resistantGlufosinate-  
resistantImidazolinone-  
resistant

Figure 2.2. Effect of POST herbicides on glyphosate-, glufosinate- and imidazolinone-resistant volunteer corn at 28 DAT. Nontreated controls are present in the back row for comparison.

## Tables

Table 2.1. Details of pre-emergence (PRE) soybean herbicides used in the study.

Herbicide	Trade name	Formulation	Rate	Manufacturer
			g ai ha <sup>-1</sup>	
Sulfentrazone + Imazethapyr	Authority Assist	480 g L <sup>-1</sup>	422	FMC Corporation, Philadelphia, PA 19103
Sulfentrazone + Chloransulam methyl	Authority First	621 g kg <sup>-1</sup>	315	Monsanto Company, 800 North Lindberg Ave., St. Louis, Mo
Sulfentrazone + Metribuzin	Authority MTZ	450 g kg <sup>-1</sup>	567	FMC Corporation
Sulfentrazone + Chlorimuron ethyl	Authority XL	700 g kg <sup>-1</sup>	343	FMC Corporation
Clomazone	Command 3ME	360 g L <sup>-1</sup>	840	FMC Corporation
Chlorimuron methyl + Flumioxazin + Thifensulfuron	Enlite	479 g kg <sup>-1</sup>	94	DuPont Crop Protection, P. Box 80705 CRP 705/L1S11, Wilmington, DE 19880-0705.
Flumioxazin + Cloransulam	Gangster co pack	510 g kg <sup>-1</sup> + 840 g kg <sup>-1</sup>	107 + 35.3	Valent USA Corporation, Walnut Creeks, CA 94596
Alachlor	Intrro	480 g L <sup>-1</sup>	2,800	Monsanto Company
Saflufenacil + Imazethapyr	Optill	680 g kg <sup>-1</sup>	95	BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709
S-metolachlor + Fomesafen	Prefix	566 g kg <sup>-1</sup>	1,490	Syngenta Crop Protection, Inc. Greensboro, NC 27419
Pendimethalin	Prowl H <sub>2</sub> O	456 g L <sup>-1</sup>	1,070	BASF Ag Products
Pendimethalin + Metribuzin	Prowl H <sub>2</sub> O + Sencor DF/Dimetric	456 g L <sup>-1</sup> + 750 g kg <sup>-1</sup>	1,070 + 420	BASF Ag Products + AgriSolutions 31832 Delhi Road Brighton, IL 62012
Imazethapyr	Pursuit	240 g L <sup>-1</sup>	70	BASF Corporation
Imazethapyr + S-metolachlor	Pursuit + Dual II Magnum	240 g L <sup>-1</sup> + 824 g kg <sup>-1</sup>	137 + 1,600	BASF Corporation + Syngenta Crop Protection
Imazaquin +S-metolachlor	Scepter + Dual II Magnum	700 g kg <sup>-1</sup> + 824 g kg <sup>-1</sup>	137 + 1,247	BASF Corporation + Syngenta Crop Protection
Metribuzin + S-metolachlor	Sencor + Dual II Magnum	750 g kg <sup>-1</sup> + 824 g kg <sup>-1</sup>	420 + 1,070	Bayer Crop Science, Research Triangle Park, NC 27709 + Syngenta Crop Protection
Trifluralin	Treflan	480 g L <sup>-1</sup>	840	Dow AgroSciences, LLC 9330 Zionsville Road Indianapolis, IN 46268
Flumioxazin	Valor SX	510 g kg <sup>-1</sup>	89	Valent U.S.A. Corporation Agricultural Products
Flumioxazin + Chlorimuron-ethyl	Valor XLT	597 g kg <sup>-1</sup>	113	Valent U.S.A. Corporation + BASF Corporation

Table 2.2. Details of post-emergence (POST) soybean herbicides used in the study

Herbicide	Trade name	Rate	Manufacturer	Adjuvant <sup>a</sup>
		g ai ha <sup>-1</sup>		
Quizalofop	Assure II	38.6	DuPont Crop Protection, P.O.Box 80705 Wilmington, DE 19880	COC 1% v/v
Fluthiacet- ethyl	Cadet	7.2	FMC Corporation, Philadelphia, PA 19103	NIS 0.25% v/v + UAN-28% 2.34 L ha <sup>-1</sup>
Imazethapyr + Glyphosate	Extreme	910	Syngenta Crop Protection, Inc. Greensboro, NC 27419	NIS 0.125% v/v + AMS 2% w/w
Fomesafen + Glyphosate	Flexstar GT	1,380	Syngenta Crop Protection	NIS 0.25% v/v + AMS 2% w/w
Fluazifop	Fusilade DX	210	Syngenta Crop Protection	NIS 0.25% v/v + UAN-28% 9.4 L ha <sup>-1</sup>
Glyphosate + Imazamox	Roundup PowerMAX + Raptor	1,120 + 44	Monsanto Company, 800 North Lindberg Ave., St. Louis, Mo	NIS 0.25% v/v + AMS 1.8% wt/wt
Glyphosate + Imazaquin	Roundup PowerMAX + Scepter	1,120 + 76	Monsanto Company + BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709	NIS 0.25% v/v
Glyphosate + Acifluorfen	Roundup PowerMAX + Ultra Blazer	1,120 + 340	Monsanto Company + United Phosphorus, Inc. 630 Freedom Business Center, PA 19406	NIS 0.25% v/v + AMS 2% wt/wt
Glufosinate	Liberty 280 SL	595	Bayer Crop Science, Research Triangle Park, NC 27709	AMS 2% wt/wt
Sethoxydim	Poast Plus	350	BASF Corporation	COC 2% v/v + AMS 2.8% wt/wt
Imazamox	Raptor	44	BASF Corporation	NIS 0.25% v/v + AMS 1.8% wt/wt
Clethodim	Select Max	136	Valent USA Corporation, Walnut Creek, CA 94596	NIS 0.25% v/v + AMS 1.8% wt/wt
Fenoxaprop + Fluazifop	Fusion	135	Syngenta Crop Protection	COC 0.25% v/v + AMS 4.5% wt/wt
Glyphosate + Chlorimuron-ethyl	Roundup PowerMAX + Classic	1,120 + 5.8	Monsanto Company + DuPont Crop Protection, P. Box 80705 CRP 705/L1S11, Wilmington, DE	NIS 0.25% v/v + AMS 2% wt/wt
Imazethapyr	Pursuit	70	BASF Corporation	NIS 0.25% v/v + AMS 1.8% wt/wt
Acifluorfen	Ultra Blazer	170	United Phosphorous Inc.	NIS 0.25% v/v + AMS 2% wt/wt
Imazamox + Acifluorfen	Raptor + Ultra Blazer	35 + 280	BASF Corporation + United Phosphorous Inc.	NIS 0.25% v/v + AMS 2% wt/wt

<sup>a</sup>Abbreviations: AMS=ammonium sulfate (DSM chemicals North America Inc., Augusta, GA), COC=crop oil concentrate (Agridex, Helena Chemical Co., Collierville, TN), NIS=nonionic surfactant (Induce, Helena Chemical Co., Collierville, TN), UAN-28=Urea ammonia nitrate solution 28% (Sylvite Agri-Services, Ontario, Canada).

Table 2.3. Effect of PRE soybean herbicides for the control of glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn at 7 and 21 DAT, cumulative emergence at 21 DAT, and volunteer corn biomass

Herbicide	Rate	Control at 7 DAT <sup>b,c</sup>			Control at 21 DAT <sup>b,c</sup>			Cumulative emergence 21 DAT <sup>c</sup>			Volunteer corn biomass <sup>c</sup>		
		Gluf <sup>a</sup>	Glyph <sup>a</sup>	Imida <sup>a</sup>	Gluf <sup>a</sup>	Glyph <sup>a</sup>	Imida <sup>a</sup>	Gluf <sup>a</sup>	Glyph <sup>a</sup>	Imida <sup>a</sup>	Gluf <sup>a</sup>	Glyph <sup>a</sup>	Imida <sup>a</sup>
	g ai ha <sup>-1</sup>	%			%			%			g pot <sup>-1</sup>		
Nontreated Control <sup>d</sup>	-	0	0	0	0	0	0	90a	100a	100a	3.3a	3.5a	3a
Sulfentrazone + Imazethapyr	422	66ab	39ab	68a	64a-f	50abc	72abc	80a	100a	90a	1bc	1.6bc	0.4bc
Sulfentrazone + Chloransulam	315	58ab	36ab	68a	65a-f	48a-d	64a-d	90a	90a	80a	0.7bc	1bc	0.5bc
Sulfentrazone + Metribuzin	567	69a	26ab	69a	70a-d	31b-f	75abc	90a	90a	80a	0.7bc	1.1bc	0.5bc
Sulfentrazone + Chlorimuron	343	55ab	35ab	66abc	68a-e	52abc	64a-d	90a	100a	70ab	0.5bc	1bc	0.3c
Clomazone	840	50ab	16ab	68a	92a	47a-e	90a	90a	90a	60ab	0.8bc	1.1bc	0.4bc
Chlorimuron + Flumioxazin + Thifensulfuron	94	32ab	3b	29a-e	4j	6f	3f	90a	100a	60ab	2.5abc	2.3abc	1.2abc
Flumioxazin + Cloransulam	107 + 35.3	43ab	6ab	58a-e	61a-g	23c-f	67a-d	90a	100a	70ab	0.6bc	1.7abc	0.5bc
Alachlor	2,800	44ab	8ab	39a-e	22g-j	4f	4f	80a	90a	60ab	1.9abc	2.6abc	1.2abc
Saflufenacil + Imazethapyr	95	9ab	1b	29a-e	26f-j	1f	12ef	90a	100a	60ab	1.6abc	2.7abc	1.4abc
S-metolachlor + Fomesafen	1,490	51ab	29ab	75a	41c-j	13c-f	81abc	80a	100a	60ab	1.3abc	2.1abc	0.3c
Pendimethalin	1,070	24ab	14ab	5cde	19h-j	2f	1f	80a	70a	70ab	2.5abc	1.9abc	1.9abc
Pendimethalin + Metribuzin	1,070 + 420	38ab	16ab	21a-e	58a-h	14c-f	30def	90a	90a	70ab	1.5abc	1.5abc	0.8bc
Imazethapyr	70	13ab	4b	4cde	29e-j	8def	1f	90a	100a	80a	2.1abc	2.1abc	2.2abc
Imazethapyr + S-metolachlor	137 + 1,600	4b	0.5b	6b-e	8ij	8ef	1f	90a	80a	60ab	2abc	2.2abc	1.3abc
Imazaquin + S-metolachlor	137 + 1,247	36ab	18ab	3de	74abc	70ab	3f	70a	90a	70ab	0.6bc	0.6c	1.4abc
Metribuzin + S-metolachlor	420 + 1,070	6b	0.5b	25a-e	47b-i	6f	32def	90a	90a	60ab	1bc	2abc	0.5bc
Trifluralin	840	5b	0.5b	3e	1j	6f	1f	80a	100a	80a	2.6ab	3ab	2.5ab
Flumioxazin	89	45ab	16ab	39a-e	40c-j	14c-f	49b-e	90a	90a	70ab	1.6abc	2.2abc	1.3abc
Flumioxazin + Chlorimuron	113	28ab	11ab	29a-e	31d-j	21c-f	44cde	90a	80a	60ab	1.3abc	1.7abc	0.6bc

<sup>a</sup> Abbreviations. Gluf=glufosinate-resistant, Glypho=glyphosate-resistant, Imida=imidazolinone-resistant.

<sup>b</sup> The data of visual control estimates were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

<sup>c</sup> Means within columns with no common letter(s) are significantly different according to Tukey-Kramer's pairwise comparison test at  $P \leq 0.05$ .

<sup>d</sup> Visual estimates of nontreated control (0%) are not included in analysis.

Table 2.4. Effect of POST soybean herbicides for the control of 2- to 3-leaf stage glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn at 7 and 28 DAT and volunteer corn biomass.

Herbicide	Rate	Control at 7 DAT <sup>b,c</sup>			Control at 28 DAT <sup>b,c</sup>			Volunteer corn biomass <sup>c</sup>		
		Glufo <sup>a</sup>	Glypho <sup>a</sup>	Imida <sup>a</sup>	Glufo <sup>a</sup>	Glypho <sup>a</sup>	Imida <sup>a</sup>	Glufo <sup>a</sup>	Glypho <sup>a</sup>	Imida <sup>a</sup>
	g ae or ai ha <sup>-1</sup>	%						g pot <sup>-1</sup>		
Nontreated Control <sup>d</sup>	-	0	0	0	0	0	0	4a	4a	4a
Quizalofop	38.6	63abc	64abc	39bcd	99a	99a	99a	1b	1b	1d
Fluthiacet-ethyl	7.2	33def	32def	28cde	12d	11ef	11ef	4a	4a	4a
Imazethapyr + Glyphosate	910	47b-e	47b-e	68a	85ab	53c	94ab	2b	2b	0.7d
Fomesafen + Glyphosate	1,380	57a-d	57a-d	57ab	70b	56c	80b	2b	2b	1.2cd
Fluazifop	210	75a	75a	45ab	99a	99a	99a	1b	1b	1d
Glyphosate + Imazamox	1,120 + 44	72ab	71ab	65a	91ab	65b	95ab	1b	1b	1d
Glyphosate + Imazaquin	1,120 + 76	49a-e	48a-e	55ab	85ab	59c	91ab	1b	1b	1d
Glyphosate + Acifluorfen	1,120 + 340	58a-d	57a-d	60ab	83ab	53c	87ab	1.5b	1b	1d
Glufosinate	595	23ef	25ef	17def	12d	65b	21cde	4a	2b	3ab
Sethoxydim	350	70ab	69ab	37bcd	97a	97a	96ab	1b	1b	1.2cd
Imazamox	44	31def	30def	9ef	57c	57c	31c	2b	2b	2bc
Clethodim	136	74ab	72ab	45abc	99a	99a	99a	1b	1b	1d
Fenoxaprop + Fluazifop	135	48a-e	50a-e	28cde	98a	98a	99a	1b	1b	1d
Glyphosate + Chlorimuron-ethyl	1,120 + 5.8	51a-d	52a-d	58ab	64b	64b	98a	1b	2b	2bc
Imazethapyr	70	5f	7f	2f	1d	1d	1f	4a	4a	3ab
Acifluorfen	170	32def	30def	28cde	10d	10d	13def	4a	4a	4a
Imazamox + Acifluorfen	35 + 280	38cde	36cd	36bcd	50c	51c	30cd	2b	2b	3ab

<sup>a</sup> Abbreviation. DAT=days after treatment; Glufo=glufosinate-resistant, Glypho=glyphosate-resistant, Imida=imidazolinone-resistant.

<sup>b</sup> The data of visual control estimates were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

<sup>c</sup> Means within columns with no common letter(s) are significantly different according to Tukey-Kramer's pairwise comparison test at  $P \leq 0.05$ .

<sup>d</sup> Visual estimates of nontreated control (0%) are not included in analysis.



Table 2.5. Effect of POST soybean herbicides for control of 5- to 6-leaf stage glufosinate-, glyphosate-, and imidazolinone-resistant volunteer corn at 7 and 28 DAT and volunteer corn biomass.

Herbicide	Rate	Control at 7 DAT <sup>b,c</sup>			Control at 28 DAT <sup>b,c</sup>			Volunteer corn biomass <sup>c</sup>		
		Glufo <sup>a</sup>	Glypho <sup>a</sup>	Imida <sup>a</sup>	Glufo <sup>a</sup>	Glypho <sup>a</sup>	Imida <sup>a</sup>	Glufo <sup>a</sup>	Glypho <sup>a</sup>	Imida <sup>a</sup>
	g ae or ai ha <sup>-1</sup>	%						g pot <sup>-1</sup>		
Nontreated control <sup>d</sup>	-	0	0	0	0	0	0	6ab	6ab	6ab
Quizalofop	38.6	8de	8de	6g	97a	97a	97a	2f	2f	3ef
Fluthiacet-ethyl	7.2	3e	3e	3g	3f	3f	4e	6ab	6ab	7a
Imazethapyr + Glyphosate	910	19b-e	19b-e	23b-f	34cde	34cde	60cd	4.5b-e	4.5b-e	4def
Fomesafen + Glyphosate	1,380	24a-d	24a-d	30a-d	44cd	45cd	55d	4b-f	4b-f	4.8b-e
Fluazifop	210	12cde	12cde	13efg	98a	97a	95a	2f	2f	2.8f
Glyphosate + Imazamox	1,120 + 44	38a	38a	42a	69bc	63bc	71a-d	2.5f	3ef	2.8f
Glyphosate + Imazaquin	1120 + 76	24a-d	24a-d	27a-e	58cd	43cd	66bcd	4b-f	4c-f	3.3def
Glyphosate + Acifluorfen	1,120 + 340	28abc	28abc	36ab	55cd	38cd	59cd	4.5b-e	4.5b-e	4c-f
Glufosinate	595	3e	3e	9fg	8ef	8ef	17e	5a-d	5a-d	5a-e
Sethoxydim	350	16b-e	16b-e	14d-g	87ab	85ab	87ab	2.7ef	2.6ef	3ef
Imazamox	44	3e	3e	3g	21def	21def	15e	5.5abc	5.5abc	6abc
Clethodim	136	18b-e	18b-e	17c-g	89a	88a	88ab	3def	3def	3ef
Fenoxaprop + Fluazifop	135	16b-e	16b-e	13efg	87ab	86ab	86ab	3def	3def	2.7f
Glyphosate + Chlorimuron-ethyl	1,120 + 5.8	33ab	33ab	33abc	50cd	44cd	65bcd	4.5b-e	4.5b-e	3ef
Imazethapyr	70	2e	2e	1g	3f	3f	1e	6ab	6ab	5.8abc
Acifluorfen	170	4e	4e	2g	4f	3f	4e	6ab	6ab	5.8abc
Imazamox + Acifluorfen	35 + 280	8de	8de	7fg	9ef	8f	10e	6ab	6ab	5a-d

<sup>a</sup> Abbreviations. DAT=days after treatment; Glufo=glufosinate-resistant, Glypho=glyphosate-resistant, Imida=imidazolinone-resistant.

<sup>b</sup> The data of visual control estimates were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

<sup>c</sup> Means within columns with no common letter(s) are significantly different according to Tukey-Kramer's pairwise comparison test at  $P \leq 0.05$ .

<sup>d</sup> Visual estimates of nontreated control (0%) are not included in analysis.

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### **Chapter 3**

## **Factors Affecting Germination and Emergence of Glyphosate-Resistant Hybrid and Volunteer Corn**

### **Abstract**

Glyphosate-resistant volunteer corn is a problematic weed in corn-soybean cropping systems, specifically in the Midwestern United States. Laboratory and glasshouse experiments were conducted in 2012 and 2013 to determine the effects of agronomic and climatic factors on germination and emergence of glyphosate-resistant hybrid and volunteer corn. Optimum germination (84 to 97%) was observed at day/night temperatures of 15/10 °C to 42.5/30 °C, while higher temperature (45/35 °C) reduced germination to < 6%. Alternating light and dark periods had no effect on germination, while germination was reduced significantly (< 65%) under increased osmotic stress (–0.4 to –1.3 MPa) with optimum germination (> 90%) at 0 to –0.3 MPa. Germination (> 90%) was observed at a wide range of salt concentrations (0 to 160 mM) with the lowest (53%) at 320 mM. Hybrid corn germination was favored by neutral to mild alkaline pH, while acidic pH favored volunteer corn germination. Seedling emergence of hybrid and volunteer corn occurred over a wide range of seed burial depth (0- to 15-cm), with optimum emergence at a depth of 0.5- to 6-cm. Hybrid corn seedling emergence reduced from 86 to 23% at 1 and 2 days of flooding, while volunteer corn emergence was 21 and 2% at 1 and 2 days of flooding, respectively. Results of this study suggest that volunteer corn can germinate and emerge in a wide range of climatic conditions.

**Nomenclature:** Corn, *Zea mays* L.; soybean, *Glycine max* (L.) Merr.

**Keywords:** Flooding duration, light, osmotic stress, pH, salt stress, seed burial depth, temperature

## **Introduction**

Germination and emergence are critical stages in weed seed establishment and persistence (Bewley and Black, 1994). Light is another important factor for the germination of many weed species (Bewley and Black, 1994). Few studies have reported the germination ecology of hybrid corn. For example, Idikut (2013) reported 41 and 31% germination of hybrid corn at 17 and 30 °C temperatures, and 24 and 12 hours (h) photoperiod, respectively. Fausey and McDonald (1985) reported reduction in corn seedling emergence after 2 days (d) of flooding. Higher corn seedling emergence was reported by Knappenberger and Koeller (2012) at the planting depth of 8- to 9-cm compared to a shallow planting (4- to 7-cm deep). Khayatnezhad and Gholamin (2011) reported reduction in germination of five corn cultivars with increasing salt stress levels from 0 to 250 millimolar (mM). Khodarahmpur (2011) observed reduction in germination of seven corn hybrids with increasing osmotic stress level. A better understanding of volunteer corn germination under different environmental and stress conditions could aid management strategies for this troublesome weed, including the development of models to predict germination or influence of agronomic factors such as seed burial depth and flooding duration on volunteer corn emergence.

Volunteer corn exposed to various environmental and agronomic conditions may respond differently under a range of environmental factors required for germination and emergence. Information is available on the factors affecting germination of hybrid corn, but scientific literature, to our knowledge, is not available for the effect of environmental and agronomic factors on the germination and emergence of volunteer corn. In addition,

information on effects of various environmental and agronomic factors on the germination and emergence of volunteer corn would be useful in developing integrated volunteer corn management programs. The objectives of this research were to evaluate (1) the germination of glyphosate-resistant hybrid and volunteer corn in response to temperature, light, osmotic stress, salt stress, and pH; and (2) the effect of seed burial depth and flooding duration on the emergence of hybrid and volunteer corn.

## **Materials and Methods**

GR hybrid corn was planted in 2012 at the South Central Agriculture Lab, University of Nebraska-Lincoln, Clay Center, NE. After harvesting, seeds were kept at room temperature until used as volunteer corn in this study. A preliminary study was conducted to determine the percent germination of hybrid and volunteer corn seeds, with the results suggesting  $\geq 98\%$  germination (data not shown). Laboratory and glasshouse experiments were conducted in 2012 and 2013 at the University of Nebraska-Lincoln, USA. Before initiating the study, hybrid and volunteer corn seeds were surface-sterilized in a 0.5% sodium hypochlorite solution for 10 to 15 minutes, and were rinsed with running tap water for 5 min. Laboratory experiments were arranged in a factorial randomized complete block design with six replications, considering type of corn (hybrid or volunteer corn) and response variables (environmental factor) as two factors. Fifteen sterilized seeds, each of hybrid and volunteer corn, were placed on a filter paper (Whatman # 4 filter paper, International Ltd., Maidstone, U.K.) in separate 9-cm petri dishes, unless stated otherwise, and 7.5 ml of distilled water was added to the petri dishes. Petri dishes were sealed with Parafilm (American National Company, Greenwich, CT 06836) to prevent desiccation during incubation. Each replication was arranged on a different shelf in the germination chamber and considered as a block. Petri dishes were kept in the germination chamber for 7 d at a day/night temperature of 30/20 °C and 12 h photoperiod, except in the study of effect of light and temperature. After 7 d, the germinated seeds were counted and converted to percent germination. Experiments to evaluate the effects of depth of sowing and flooding duration on the emergence of



glyphosate-resistant hybrid and volunteer corn were conducted under glasshouse conditions in a factorial completely randomized block design with four replications. All experiments were repeated once.

### **Effect of temperature**

Germination of hybrid and volunteer corn seeds was determined in a growth chamber under eight fluctuating day/night temperature regimes of 12.5/7.5, 15/10, 20/12.5, 30/20, 37.5/25, 42.5/30, and 45/35 °C. Photoperiod was set at 12 h (day/night).

### **Effect of light**

Light regimes consisted of complete dark (24/0 h dark/light), complete light (0/24 h dark/light), and alternating light and dark conditions (4/20, 8/16, 12/12, 16/8, or 20/4 h dark/light). During this experiment, a constant day/night temperature of 30/20 °C was maintained in the germination chamber.

### **Effect of osmotic stress**

Solutions with the osmotic potential of 0, -0.3, -0.4, -0.6, -0.9, and -1.3 MPa were prepared by dissolving 0, 154, 191, 230, 297, and 350 g of polyethylene glycol (PEG; polyethylene glycol 8000, Fisher Scientific, Fair Lawn, NJ 07410) in 1 L of deionized water (Michel, 1983; Shaw et al., 1991). Petri dishes were placed in the germination chamber and maintained at a constant day/night temperature of 30/20 °C.

### **Effect of salt stress**

Sodium chloride (NaCl; Fisher Scientific, Fairlawn, NJ 07410) solutions of 0, 10, 20, 40, 80, 160, and 320 mM were prepared and were used as a germination media (Michel, 1983). A solution of NaCl (7.5 ml) was added to each petri dish and was placed

in the germination chamber with a maintained day/night temperature of 30/20 °C and 12 h photoperiod.

### **Effect of pH**

Buffer solutions with pH levels of 3, 4, 5, 6, 7, 8, and 9 were prepared according to the method described by Gortner (1949) and Shaw et al. (1987). 0.1 Molar (M) potassium hydrogen phthalate (Fisher Scientific, Fairlawn, NJ 07410) was used to obtain pH solutions of 3, 4, 5, and 6; while 25 mM sodium borate (Fisher Science Education, Hanover Park, IL 60133) was used to obtain pH solutions of 7, 8, and 9. Deionized water was used as a germination medium for comparison. 7.5 ml of these buffer solutions was added to the petri dishes, which were then placed in the germination chamber for 7 d with a day/night temperature maintained at 30/20 °C.

### **Effect of Seed burial depth on seedling emergence**

Four replicates with twenty seeds of GR hybrid and volunteer corn were planted at depths of 0, 0.5, 1, 2, 4, 6, and 10 cm below the soil surface in 20-cm deep and 9-cm diam plastic pots. In addition, to evaluate the effect of deeper burial depths on germination, experiments were conducted in the large size pots. Twenty seeds were planted at depths of 15- and 20-cm in 24-cm deep and 11-cm diameter plastic pots, and fifteen seeds were planted at depths of 25-cm in 60-cm deep and 10-cm diam. plastic pots filled with 80% soil collected from a field in Nebraska and 20% commercial potting mix (Berger BM1 potting mix, Berger Peat Moss Ltd., Quebec, Canada). The experiment was conducted under glasshouse conditions with day/night temperature maintained at  $25 \pm 5/20 \pm 5$  °C. Pots were initially subsurface irrigated to field capacity and then surface

irrigated daily to maintain the adequate soil moisture. Emerged seedlings were counted every 7, 14, and 21 d after planting. Seedlings were considered emerged when two cotyledons could be visually discerned, and emerged seedlings were removed after weekly counts.

### **Effect of flooding duration on seedling emergence**

Four replicates of twenty five GR hybrid and volunteer corn seeds were planted 4 cm deep in a separate plastic pot (23-cm deep and 24-cm diam.) filled with 80% of the soil (as described above ) and 20% of commercial potting mix. Results of the seed burial depth study indicated that maximum germination occurred when seeds were buried at 4 cm. Flooding duration treatments were 0, 1, 2, 4, 7, 14, and 21 d. Water was maintained 2 cm above the soil surface for above mentioned period to stimulate flooding. After exposure to a given period of flooding, the excess water was drained by poking holes on the sides of the pots. The emerged seedlings were counted at 7, 14, 21, and 35 d after planting. Glasshouse conditions were the same as in the seed burial depth experiment.

Data analysis was performed using PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC). Percent germination data were arcsine square-root transformed before analysis; however, back-transformed data are presented with mean separation based on transformed data. A preliminary data analysis suggested no significant difference between experimental runs. Treatments and corn types (hybrid and volunteer) were considered fixed effects, while replications and experimental runs were considered random effects in the model. Regression analysis was used where appropriate; otherwise, means were separated using Tukey-Kramer's pairwise comparison test at  $P \leq$

0.05. Percent germination values at different osmotic concentrations were best fitted to a three- parameter sigmoid model using Sigma Plot version 10.0 (Systat Software Inc., San Jose, CA 95110). The model fitted was:

$$G (\%) = G_{\max} / \{ 1 + \exp[-(x - x_{50})/G_{\text{rate}}] \} \quad [1]$$

where G represents the total germination (%) at an osmotic concentration x,  $G_{\max}$  represents the maximum germination (%),  $x_{50}$  represents the osmotic potential required to inhibit 50% of the maximum germination, and  $G_{\text{rate}}$  indicates the slope. A polynomial quadratic model was fitted to the percent germination values obtained at different salt concentrations. The model fitted was:

$$G (\%) = G_{\max} + ax - bx^2 \quad [2]$$

where G represents the total germination (%) at salt concentration x,  $G_{\max}$  represents the maximum germination (%), and a, and b are the model parameters.

## Results and Discussion

### Effect of Temperature

No significant two-way interaction for germination was observed among the corn types and temperature treatments (P-value = 0.9506); therefore, combined data are presented. Different day and night temperatures affected the seed germination (P-value < 0.0001) (Figure 3.1). Optimum germination (84 to 97%) was observed at a day/night temperature of 15/10 to 42.5/30 °C, whereas the lowest germination (6%) was observed at 45/35 °C with a 12 h photoperiod. At the lowest day/night temperature (12.5 /7.5 °C), the germination reduced to 62%; however, it was comparable with 15/10, 20/12.5, 37.5/30, and 42.5/30 °C (Figure 3.1). Germination of GR hybrid and volunteer corn was reported over the fluctuating day/night temperature regime tested. The optimum germination was reported at four fluctuating day/night temperatures ranging from 15/10 to 42.5/30 °C than at the highest (45/35 °C) temperature tested Bolfrey-Arku et al. (2011) reported higher germination of the two populations of *Rottboellia cochinchinensis* (Lour.) W.D. Clayton (itchgrass) at an intermediate fluctuating day/night temperature (25/15 °C) with a 12 h photoperiod compared to the lowest fluctuating temperature regimes of 20/10 °C, but it was comparable with 30/20 and 35/25 °C day/night fluctuating temperatures. In contrast, Idikut (2013) reported a significant effect of temperature (17 and 30 °C) on the germination of three corn varieties. Results suggested that similar to the hybrid corn, volunteer corn can germinate over a wide range of day/night temperatures. The 30-year average temperature for spring and summer months in Nebraska ranged from 9.2 to 22.2

°C, respectively (NOAA-NCDC, 2014), suggesting the ability of volunteer corn to survive cold temperatures in winter and germinate during spring and summer months.

### **Effect of Light**

Germination was not affected by corn type, light conditions, and the interaction among corn type and light conditions; therefore combined data of hybrid and volunteer corn are presented (Figure 3.2). At a constant day/night temperature (30/20 °C), > 90% germination was observed under complete dark (24/0 h dark/light), complete light (0/24 h dark/light), and alternate light and dark conditions (4/20, 8/16, 12/12, 16/8, or 20/4 h dark/light). GR hybrid and volunteer corn are negatively photoblastic, because neither complete light or dark conditions, nor their alternate regimes had any effect on germination. Similarly, Norsworthy and Oliveira (2006) reported no effect of light on the germination of *Senna obtusifolia* L. (sicklepod). In contrast, Idikut (2013) reported a significant difference in the germination of three corn varieties at complete light compared to 12/12 h dark/light conditions. Bolfrey-Arku et al. (2011) reported that light was not a requirement for germination of two *R. cochinchinensis* populations; however, a light/dark regime stimulated germination by 96%, across temperatures and populations. Some species provides higher germination in alternate light/dark cycle compared to dark conditions. For example, Chauhan and Johnson (2009) reported higher germination (43%) of *Echinochloa colona* (junglerice) at alternate light/dark regimes compared to the dark regimes (4%). As light has no effect on the germination of volunteer corn, higher germination rates would not only restrict to the surface dropped seeds but also to the seeds present deep in the soil. Germination can occur in the absence of the light but

depends on the amount of food reserve present in the seeds to help plants emerge out of deeper depths. Thus, crop canopy and residues present in the field will not play an important role in reducing germination of volunteer corn.

### **Effect of Osmotic Stress**

A non-significant two-way interaction among the corn types and osmotic stress levels was observed (P-value = 0.2156). Highest germination (> 90%) was observed at lower osmotic stress level of 0 to –0.3 Megapascal (MPa), whereas it was lowest ( $\leq 5\%$ ) at higher osmotic stress levels (–0.9 to –1.3 MPa) (Figure 3.3). Germination was reduced to 63 and 36% as osmotic stress increased to –0.4 and –0.6 MPa, respectively. Highest germination was observed at lower osmotic stress level whereas it was lowest at higher osmotic stress level. These results were similar to those obtained by Khodarahmpur (2011), who reported the lowest germination ( $\leq 23\%$ ) of the seven corn hybrids at lower osmotic stress levels (–0.9 MPa to –1.2 MPa) compared to the untreated control. Similarly, Chejara et al. (2008) reported reduced germination (93 to 43%) of *Hyparrhenia hirta* (L.) Stapf. (coolatai grass), with increasing water stress level from 0 to –0.37 MPa. The osmotic range for volunteer corn germination is narrow but it could germinate under mild drought conditions.

### **Effect of Salt Stress**

Analysis of variance suggested no significant interaction among the corn types and different salt stress levels (P-value = 0.4285). Germination was > 90% at salt stress level of 0 to 160 mM (Figure 3.4). Germination of the hybrid and volunteer corn was reduced to 53% at the highest salt stress level tested (320 mM). Higher germination was

reported at salt stress level of 0 to 160 mM. Similarly, Idikut (2013) reported no difference in the germination of three corn hybrids at the salt stress level of 0 to 100 mM. Germination of the hybrid and volunteer corn was reduced to 53% at the highest salt stress level (320 mM). So, volunteer corn is not very sensitive to saline conditions; thus, providing information for its germination and emergence in salt affected soils. In contrast, Carpıcı et al. (2009) reported a linear decrease in the germination of different corn cultivars at salt stress level of 0 mM (55%) to 250 mM (23%). Khayatnezhad and Gholamin (2011) also reported a linear decrease in the germination of five corn cultivars (53 to 21%) with increasing salt concentrations (0 to 250 mM). This indicates the variation in germination response of different corn varieties to different salt stress levels.

### **Effect of pH**

Germination was influenced by the corn type, pH of the germination solution, and interaction of the corn type and pH (P-value = 0.0054). Germination of volunteer corn was 86 and 88% compared to 55 and 75% germination of hybrid corn at a pH of 5 and 6, respectively (Figure 3.5). The highest germination of hybrid corn (82 to 85%) was observed at neutral to slightly alkaline pH (7 to 8), whereas the germination of volunteer corn was 74 to 78% at these pH values. At highly acidic pH (3 and 4), volunteer corn germination was 47 and 68%, respectively, but hybrid corn germination was  $\leq 5\%$  indicating germination advantage for volunteer corn under highly acidic pH. At a highly alkaline pH (9), the germination of hybrid and volunteer corn was similar (62 to 66%). Volunteer corn was more tolerant to acidic pH (3 to 6), while hybrid corn showed tolerance to the alkaline pH (8 to 9). GR volunteer corn can germinate over a wide range



of pH (3 to 9) compared to glyphosate-resistant hybrid corn (5 to 9). Similarly, Chauhan and Johnson (2008) reported optimum germination (92 to 95%) of *Eleusine indica* (L.) Gaertn. (goosegrass) at a pH range of 5 to 10. Volunteer corn was more tolerant to acidic pH (3 to 6), while hybrid corn showed tolerance to the alkaline pH (8 to 9). Ramirez et al. (2014) reported better germination (49 to 79%) of *Citrullus lanatus* (Thunb.) Matsumura & Nakai var. *citroides* (Bailey) Mansf. (citron melon) at acidic to neutral pH (3 to 7) compared to alkaline pH ( $\leq 5\%$ ). Additionally, most agricultural soils in Nebraska are in the pH range (5 to 8) in which volunteer corn germination was 74 to 88%.

### **Effect of Seed Burial Depth on Seedling Emergence**

The effect of varying seed burial depths on seedling emergence of hybrid and volunteer corn was significant (P-value < 0.001). The highest seedling emergence (> 87%) of hybrid and volunteer corn was observed at 0.5- to 6-cm burial depth without difference among them (Figure 3.6). The emergence was slightly reduced when seeds were sown on the surface of the soil; however, 80 to 84% emergence was observed. However, in this study, the emergence of hybrid and volunteer corn was 84 and 53%, respectively, even at the burial depth of 15 cm. Seedling emergence was < 20 and 0% at 20- and 25-cm burial depth, respectively. GR hybrid and volunteer corn germinated up to 15-cm planting depth while, the highest seedling emergence was observed at 0.5- to 6-cm burial depth. Andrew (1953) reported no significant difference in the emergence of sweet corn strains at 2.5- (84%) and 10-cm (83%) burial depths. The emergence was slightly reduced when seeds were sown on the surface of the soil. This indicates that corn seeds lost during harvest and present on the surface of soil may emerge in spring if they survive

winter, and are not subjected to predation. Therefore, it is likely that adoption of no-tillage system may have increased survival and occurrence of volunteer corn in the Midwestern United States. Chauhan et al. (2006) reported reduction in the emergence (44 to 0%) of *Lolium rigidum* Gaudin (rigid ryegrass) with increasing seed burial depth (1- to 10-cm), with reduced emergence (16%) from the seeds sown on the soil surface. Seed reserve can be a factor in seedling emergence behavior at increasing sowing depths (Mennan & Ngouajio, 2006), as can weather and soil characteristics (Benvenuti & Macchia, 1997). Thus, tillage practices are not the key agronomic practices to help control volunteer corn as the seeds incorporated deep in the soil could still emerge from 15 cm soil depth.

### **Effect of Flooding Duration on Seedling Emergence**

Effect of flooding duration treatments on the seedling emergence of hybrid and volunteer corn was significant ( $P\text{-value} < 0.0001$ ). Seedling emergence of hybrid corn was not affected by 1 d of flooding and it was comparable with no flooding treatment; however, volunteer corn emergence was  $\leq 20\%$  (Figure 3.7). A reduction in hybrid corn seedling emergence to 23% was observed at 2 d of flooding, while volunteer corn emergence reduced to 2%. At 4 d of flooding duration,  $< 5\%$  emergence was observed for hybrid and volunteer corn. No seedling emergence was observed beyond 4 d of flooding, indicating sensitivity of both the corn types to excess water conditions continuously for 4 d or more. Volunteer corn was more sensitive to flooding compared to the hybrid corn during first day of flooding. A reduction in emergence was reported for hybrid and volunteer corn with increase in flooding duration up to 4 days. Similarly, Fausey and

McDonald (1985) reported significant reduction in hybrid corn seedling emergence to 36 and 1% after 2 and 6 d of flooding, respectively. King and Grace (2000) reported reduced germination (12%) of *Imperata cylindrical* (L.) Beauv. (cogongrass) in flooded conditions compared to the saturated (50%). This indicates that flooding could be a limiting factor for germination and emergence of volunteer corn. Thus, emergence of volunteer corn would be restricted under more than expected rainfall conditions that result in water logged conditions at least for 2 d or in poorly drained soils.

This is the first report describing factors affecting germination and emergence of glyphosate-resistant hybrid and volunteer corn. Results confirmed that increasing prevalence of glyphosate-resistant volunteer corn in the Midwestern United States can not only be correlated with increased adoption of glyphosate-resistant corn, but also to favorable environmental factors. On the other hand, no pre-emergence herbicide is currently available that effectively controls glyphosate-resistant volunteer corn in soybean (Chahal et al., 2014), therefore, control of this problem weed is totally depended on post-emergence application of Acetyl Co-A carboxylase (ACCase) inhibiting herbicides (Deen et al., 2006; Marquardt & Johnson, 2013). This information can be used to develop an integrated volunteer corn management program based on biology, germination ecology, use of improved agronomic practices, herbicide-resistant corn traits, and use of herbicides in corn-soybean cropping systems.

### **Limitation of Research Project**

The temperature or light conditions are usually not the same for each day in a season. In the effect of temperature and light study, similar conditions were maintained in

the growth chamber according to the treatment for one week; thus, not representing the actual field conditions.

### **Future Directions**

In the future, more environmental and agronomic factors representing the actual field conditions could be studied. Temperature treatments lower than 12.5/7.5 C day/night temperature could also be considered.

### Figures

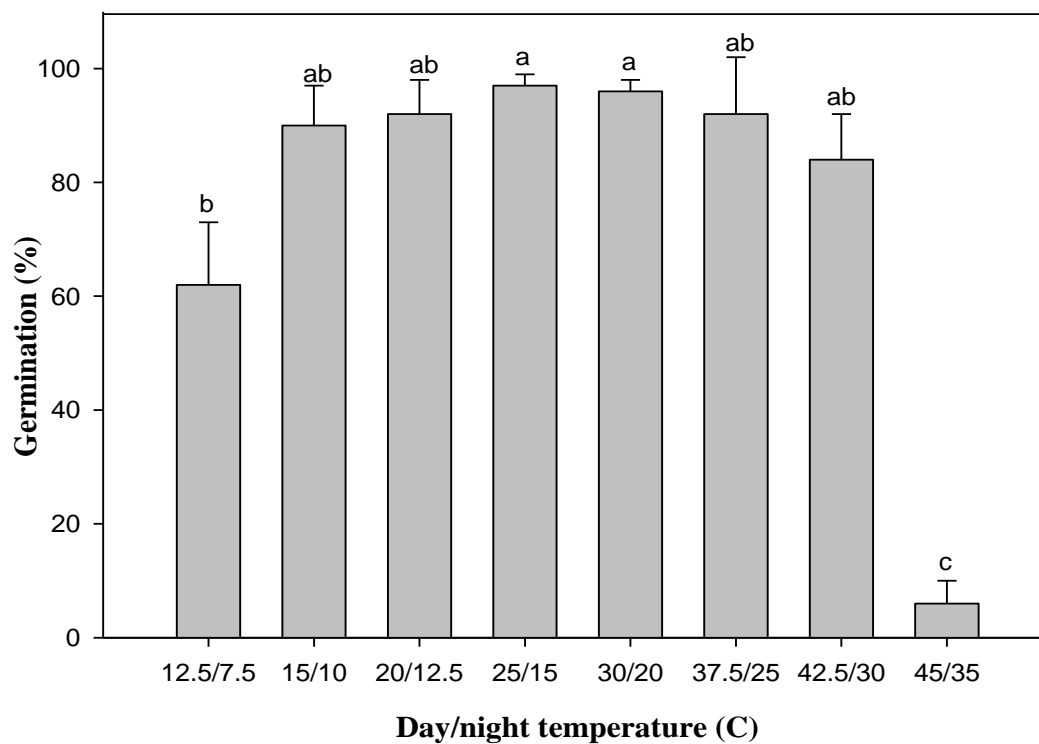


Figure 3.1. Germination of glyphosate-resistant hybrid and volunteer corn under varying day/night temperatures. No significant difference was observed for germination between hybrid and volunteer corn, therefore data were combined. Bars with same letters are not significantly different at  $\alpha = 0.05$ . Abbreviation: C, degree Celsius.

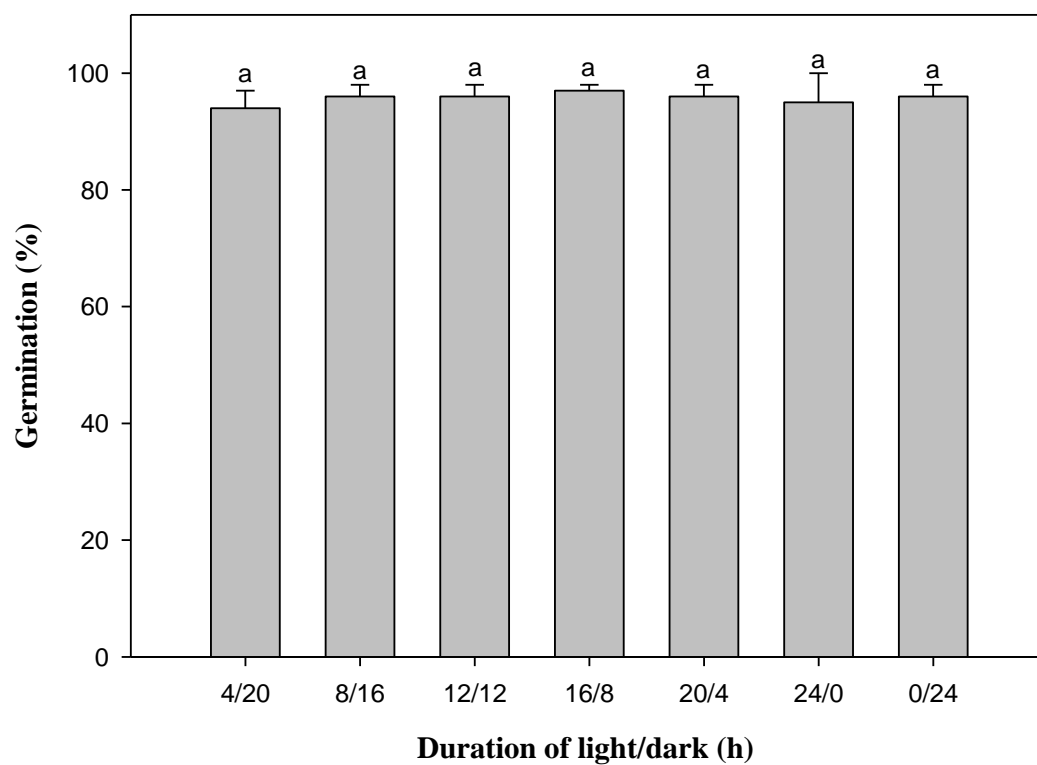


Figure 3.2. Germination of glyphosate-resistant hybrid and volunteer corn under varying light and dark conditions. No significant difference was observed for germination between hybrid and volunteer corn, therefore data were combined. Bars with the same letter are not significantly different at  $\alpha = 0.05$ . Abbreviation: h, hours.

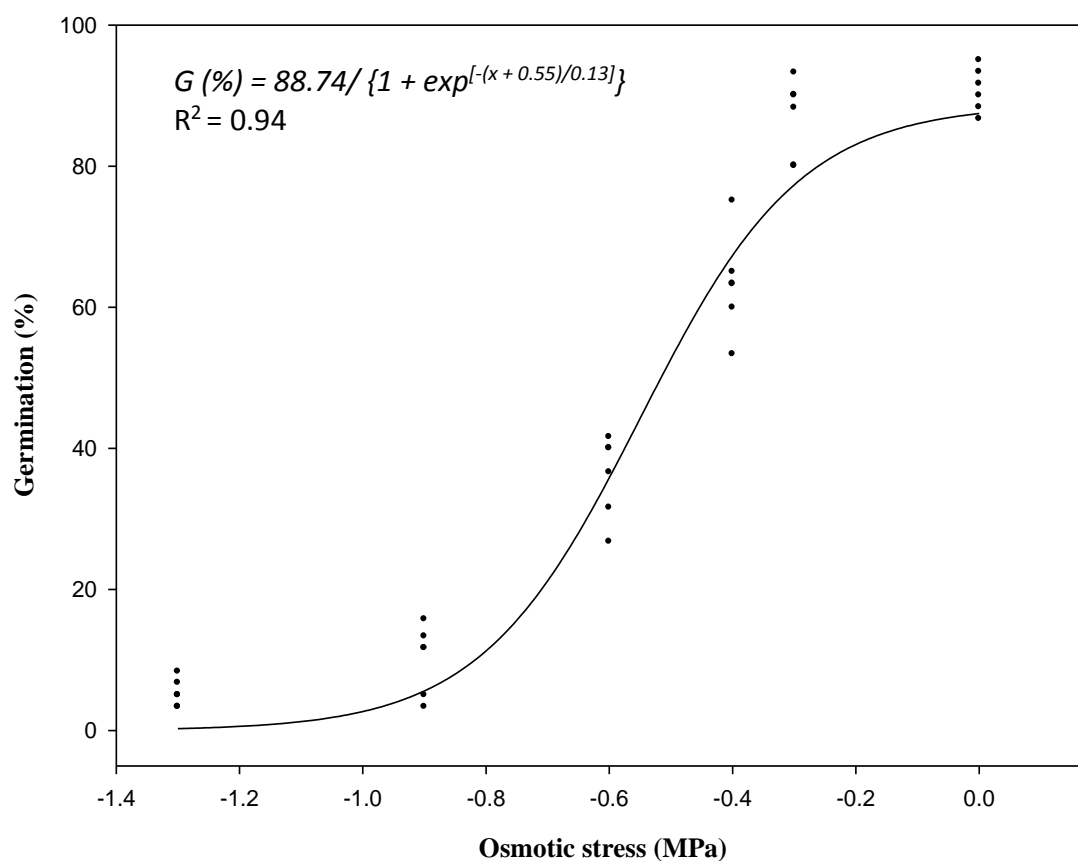


Figure 3.3. Germination of glyphosate-resistant hybrid and volunteer corn under varying osmotic stress conditions. No significant difference was observed for germination between hybrid and volunteer corn, therefore data were combined. Abbreviation: G, germination; MPa, megapascal.

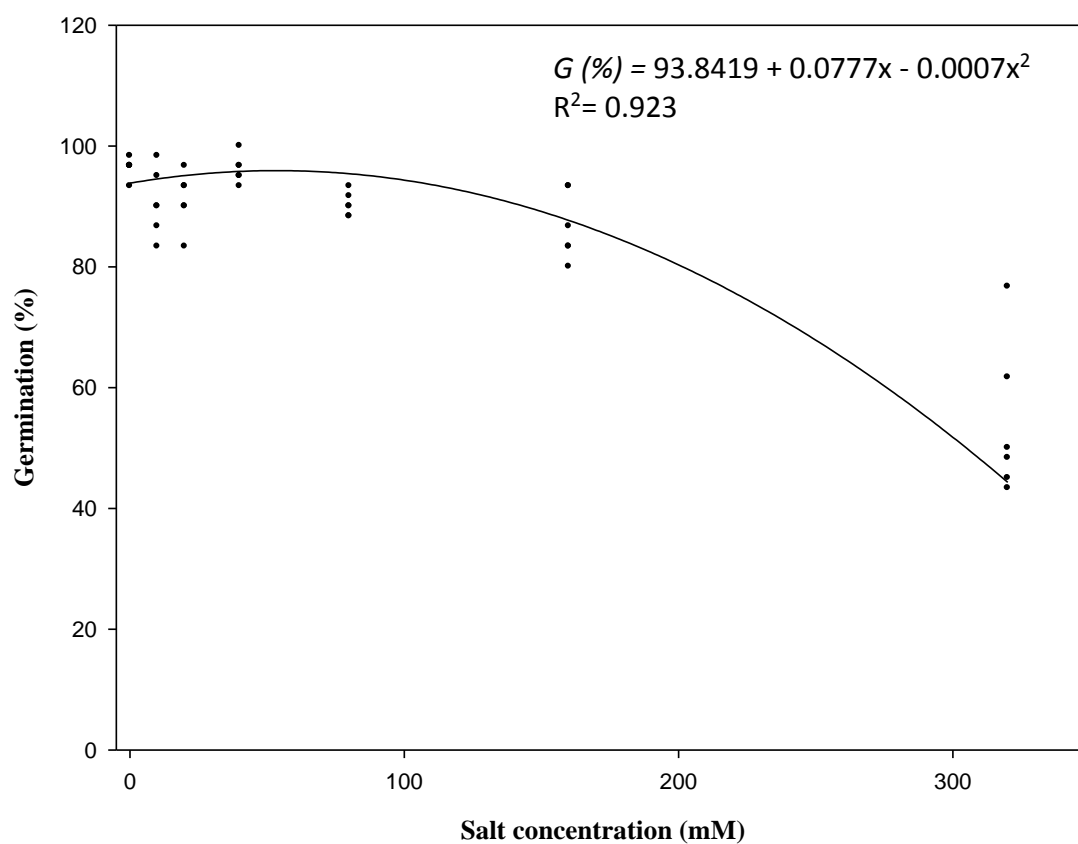


Figure 3.4. Germination of glyphosate-resistant hybrid and volunteer corn at different salt concentrations after 1 week of incubating at day/night temperature of 30/20 C and 12 h photoperiod. No significant difference for germination was observed between hybrid and volunteer corn, therefore data were combined. Abbreviation: mM, millimolar.



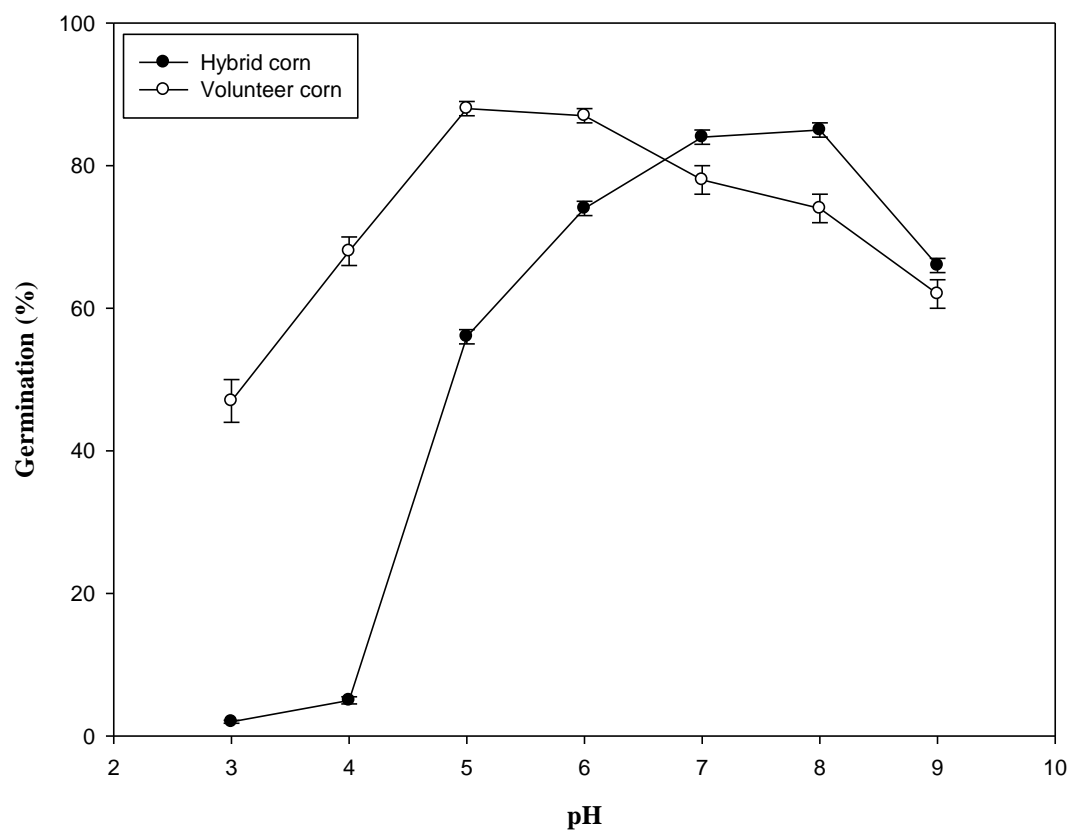


Figure 3.5. Germination of glyphosate-resistant hybrid and volunteer corn at varying pH levels at constant day/night temperature of 30/20 C. Horizontal bars are standard errors of the mean.

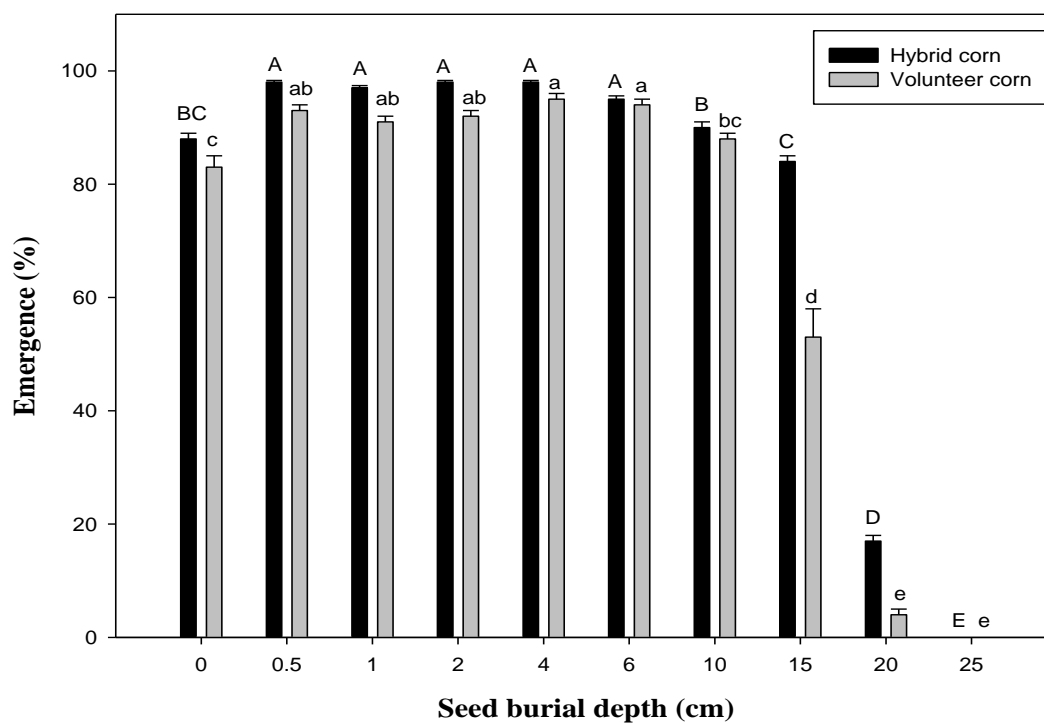


Figure 3.6. Seedling emergence of glyphosate-resistant hybrid and volunteer corn at varying seed burial depths. Bars with the same letter(s) are not significantly different at  $\alpha = 0.05$ . Capital letters represent comparison among hybrid corn and small letters represent comparison among volunteer corn. Abbreviation: cm, centimeter.

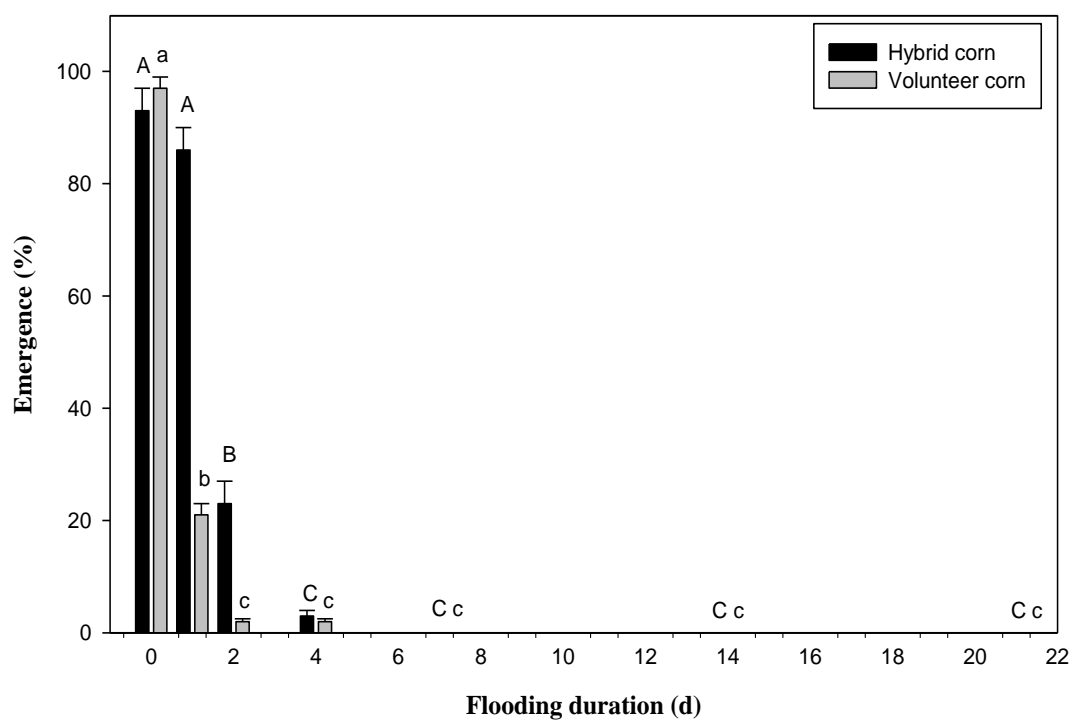


Figure 3.7. Seedling emergence of glyphosate-resistant hybrid and volunteer corn at different flooding durations. Bars with the same letter(s) are not significantly different at  $\alpha = 0.05$ . Capital letters represent comparison among hybrid corn and small letters represent comparison among volunteer corn. Abbreviation: d, day (s)

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## **Chapter 4**

### **Impact of Glyphosate-Resistant Volunteer Corn Density, Control Timing, and Late Season Emergence on Glyphosate-Resistant Soybean Yields**

#### **Abstract**

Glyphosate-resistant volunteer corn is a troublesome weed of soybean in a corn-soybean rotation as well as in a continuous corn production system. Volunteer corn can be effectively controlled with the application of acetyl-coenzyme A carboxylase-(ACCase) inhibitors. Majority of growers control volunteer corn when it is visible above the soybean canopy, but that results in early season competition with soybean. Soybean yield could be improved by identifying the critical period for controlling volunteer corn emerging early and late in the season. The objectives of this study were to evaluate the impact of different densities of glyphosate-resistant volunteer corn present as individual plant or clump at different control timings, and late season volunteer corn emergence after being controlled at different soybean growth stages on soybean yields. Field experiments were conducted under irrigated conditions at the South Central Agricultural Laboratory (SCAL), University of Nebraska-Lincoln, near Clay Center, NE and under rainfed conditions at Havelock Farm, University of Nebraska-Lincoln, NE in 2013 and 2014. To maintain desired isolated volunteer corn plants (1,250, 2,500, 5,000, and 10,000 plants ha<sup>-1</sup>) and clumps densities (63, 125, 250, and 500 ha<sup>-1</sup>), individual seeds and whole ears were hand planted in each plot based on their respective target densities. Volunteer corn was controlled with application of clethodim at V4, V6, or R2 soybean growth

stages. Late season volunteer corn emergence had no significant effect on the soybean yield with all volunteer corn densities and control timings at both locations in 2013 and 2014. During first year of study at Clay Center, no significant effect of different volunteer corn densities and control timings was observed on soybean yield. Lower soybean yield was reported at the highest isolated volunteer corn plants (10,000 plants ha<sup>-1</sup>) plus clump density (500 clumps ha<sup>-1</sup>) left uncontrolled or controlled at R2 soybean growth stage during second and both years of study at Clay Center and Lincoln, respectively. Although no yield reduction was reported with lower volunteer corn densities ( $\leq 5,000$  plants ha<sup>-1</sup>) at all control timings, control is necessary to avoid interference of volunteer corn during harvesting operations and attraction of western corn rootworm.

**Nomenclature:** Clethodim; soybean, *Glycine max* (L.) Merr.; volunteer corn, *Zea mays* L.

**Keywords:** Control timing, density, herbicide-resistant, late-season emergence, weed control.



## Introduction

Volunteer corn density plays an important role in reducing soybean yield by providing competition throughout the growing season, if not controlled. Clumps of volunteer corn plants cause more soybean yield loss compared to individual plants. Andersen et al. (1982) reported reduction in soybean yield from 31 to 83% with increase in volunteer corn clump density from 1 to 4 clumps spaced every 2.4 m of soybean row. Management of volunteer corn is challenging due to the fact that PRE, soil applied herbicides registered in soybean are not very effective (Beckett and Stoller, 1988) and provides only partial control (Chahal et al., 2014). Therefore, only option to control volunteer corn in soybean is POST application of acetyl-coenzyme A carboxylase (ACCase) inhibiting-herbicides (Beckett and Stoller, 1988; Beckett et al., 1992; Chahal et al., 2014; Deen et al., 2006; Marquardt and Johnson, 2013; Young and Hart, 1997). Indeed, majority of growers control volunteer corn when it is visible above the soybean canopy, but that results in early season competition with soybean.

Soybean yield could be improved by identifying the critical period for controlling volunteer corn emerging early and late in the season. Critical period of weed control in soybean is longer under no-till system starting from VC (unrolled unifoliate leaves) or V1 (1<sup>st</sup> trifoliate) to R1 or beginning flowering stage (Halford et al., 2001), compared to conventional tillage system (VC to V4) at 2.5% yield loss (Van Acker et al., 1993). Volunteer corn plants emerging late could provide competition to soybean and results in yield loss. Effect of different volunteer corn densities on soybean yield has been discussed in the literature (Andersen et al., 1982; Stoller et al., 1987; Wilson et al., 2010).

However, scientific literature is not available about integrated effect of volunteer corn densities, control timings, and late season emergence of volunteer corn on yield of soybean. There is a need to identify control timing of volunteer corn, present at different densities, to nullify the effect of late season emerging volunteer corn on soybean yields. The objectives of this study were to find out the impact of 1) different densities of volunteer corn present as individual plant or clump at different control timings, and 2) late season volunteer corn emergence after being controlled at different soybean growth stages on soybean yields.

## Materials and Methods

Field experiments were conducted at two locations in 2013 and 2014 at the South Central Agricultural Laboratory (SCAL), Clay Center, NE and at Havelock Farm, University of Nebraska-Lincoln, Lincoln, NE. The soil texture at Clay Center was silty clay loam with pH of 6.5, 17% sand, 58% silt, 25% clay, and 2.5% organic matter and at Lincoln was Silty clay loam with pH of 5.6, 19% sand, 54% silt, 27% clay, and 3% organic matter. The experimental site at Clay Center was established under irrigated conditions and at Lincoln under rainfed/dryland conditions. Glyphosate-resistant soybean (Cv. 'Fontanelle 64R 20') was drilled in rows spaced 76-cm apart at a rate of 375,000 seeds ha<sup>-1</sup> at Clay Center (June 4, 2013 and May 19, 2014) and Lincoln (June 17, 2013 and May 17, 2014). To maintain desired isolated volunteer corn plants (1,250, 2,500, 5,000, and 10,000 plants ha<sup>-1</sup>) and clumps densities (63, 125, 250, and 500 ha<sup>-1</sup>), individual seeds and whole ears were hand planted in each plot based on their respective target densities at Clay Center (June 13, 2013 and May 25, 2014) and Lincoln (June 21, 2013 and May 23, 2014). A nontreated control without volunteer corn seeds and ears planted was included for comparison.

The plot size at Clay Center and Lincoln was 3 x 13 m and 3 x 15 m, respectively, and the treatments were replicated four times. Split-split plot experimental design was used in this study with volunteer corn density treated as main plot. The split-plot was volunteer corn control timings depending on soybean growth stages and split-split plot was late season volunteer corn emergence. In split-plot, volunteer corn was allowed to compete with soybean until harvest or was controlled at V4, V6, or R2 soybean growth

stages by application of clethodim (Select Max, Valent USA Corporation, Walnut Creek, CA 94596) at 76 g ai ha<sup>-1</sup> at V4 stage and 136 g ai ha<sup>-1</sup> at other soybean growth stages. In the split-split plot, volunteer corn plants that emerged after clethodim treatments were allowed to grow in one split until harvest and in the second split, plants were removed two weeks later. Volunteer corn plants were 7- to 10-cm, 17- to 23-cm, and 45- to 60-cm tall at Clay Center, and 5- to 8-cm, 14- to 17-cm, and 40- to 52-cm tall at Lincoln in 2013 and 2014, when treated at V4, V6, and R2 soybean growth stages, respectively.

To minimize competition from other grass and broadleaf weeds, S-metolachlor (Dual-II Magnum, Syngenta Crop Protection, Greensboro, NC 27419) at 1.63 kg ai ha<sup>-1</sup> and glyphosate (Touchdown, Syngenta Crop Protection) at 1.06 kg ae ha<sup>-1</sup> plus AMS at 2.5% wt/v was applied preplant (2 days before soybean planting). Glyphosate was applied POST at Clay Center (July 10, 2013 and June 20, 2014) and Lincoln (July 7, 2013 and June 23, 2014) to avoid in-season competition of other grass and broadleaf weeds. All the herbicide applications were made by using a CO<sub>2</sub>-pressurized backpack sprayer consisting of a four nozzle boom fitted with AIXR 11015 flat-fan nozzles (TeeJet, Spraying Systems Co., P. O. Box 7900, Wheaton, IL 60189), and was calibrated to deliver 140 L ha<sup>-1</sup> at 276 kPa.

Soybean and volunteer corn plants were considered as emerged when a cotyledon and the first true leaf was visible, respectively, and timings were recorded. Growth stages of soybean were carefully observed at regular intervals from time of its emergence until the last application of clethodim at R2 or full flowering stage to control volunteer corn at desired soybean growth stages (V4, V6, or R2). Volunteer corn density was recorded

from two randomly selected 0.25 m<sup>2</sup> quadrats and height was measured during clethodim application. On farm weather station was used to track daily minimum and maximum temperatures, precipitation, solar radiation, and humidity. Soybean was harvested at maturity with a small-plot combine and yields were adjusted to 13% moisture content. Soybean yield components were measured on a subsample of plants from each plot.

The PROC GLIMMIX procedure of SAS version 9.3 (SAS Institute Inc, Cary, NC) was used for data analysis. Soybean yield was separated by site (Clay Center and Lincoln) due to significant interaction between sites. No significant year-by-treatment interaction for soybean yield was observed for Lincoln site; therefore, treatments including volunteer corn densities, control timing, and late-season emergence were considered as the fixed effects, while year (nested within replication) was considered a random effect. Year-by-treatment interaction for soybean yield at Clay Center was significant; therefore, yield data of both years were analyzed separately. Treatments and years were considered fixed effects in the model, whereas replication was a random effect. Where the ANOVA indicated treatment effects were significant, means were separated at  $P \leq 0.05$  using Tukey-Kramer's pairwise comparison test.

## Results and Discussion

Late season volunteer corn emergence had no effect on soybean yield at Clay Center (P-value = 0.2228) and Lincoln (P-value = 0.2018) in 2013 and 2014; therefore, data were combined (Table 4.1). Clethodim applied at V4, V6, or R2 soybean growth stages provided > 90% and > 80% control of different densities of individual volunteer corn plants and clumps, respectively, at 21 DAT. Similarly, Marquardt and Johnson (2013) reported no difference in control of different densities of volunteer corn plants with clethodim applied early or late in the season.

No significant effect of volunteer corn densities and their control timings was observed at Clay Center in 2013, partially due to hail and storm damage before harvesting. In 2014, significant reduction in soybean yield was observed at highest density of volunteer corn (10,000 plants ha<sup>-1</sup>) combined with 500 clumps ha<sup>-1</sup>, when left uncontrolled (4,994 kg ha<sup>-1</sup>) or controlled at R2 soybean stage (5,068 kg ha<sup>-1</sup>), while volunteer corn densities ≤ 5,000 plant ha<sup>-1</sup> as well as clumps ≤ 250 ha<sup>-1</sup> had no effect on yield, irrespective of the control timings. Soybean yield reduction at highest volunteer corn density with respect to control timings was also observed at Lincoln site during both years. Marquardt et al. (2013) reported no significant difference in soybean yield at different densities of volunteer corn controlled early or later in the season. Density of a weed competing for entire season is an important factor for soybean yield loss (Stoller et al., 1987). Therefore, longer volunteer corn interference period at higher densities might have contributed to the yield loss in soybean in this study.

Volunteer corn populations in the field usually composed of isolated as well as

clumps of several plants, but clumps are usually more competitive at a particular density than individual plants (Andersen et al., 1982). Beckett and Stoller (1988) reported soybean yield loss of 21 and 51% at volunteer corn density of 5,380 and 10,760 clumps  $\text{ha}^{-1}$ , respectively. Volunteer corn clump densities maintained in this study were  $\leq 500$  clumps  $\text{ha}^{-1}$ ; therefore, clumps along with individual plants did not play an important role to cause soybean yield reduction, except at highest volunteer corn isolated plant density (10,000 plants  $\text{ha}^{-1}$ ) combined with the highest number of clumps (500  $\text{ha}^{-1}$ ). Most of the late emerging volunteer corn population after being controlled at different control timings were comprised of clumps rather than individual plants (data not shown). This might have accounted for lower response of soybean to late emerged volunteer corn in terms of yield as more competition could have been expected at higher volunteer corn clump densities. Under no-till condition, as maintained in this study, critical period of weed control in soybean is longer (VC to R1) compared to conventional tillage (V1 to V4) (Halford et al., 2001; Van Acker et al., 1993). In contrast, no effect of volunteer corn competition at lower densities was observed on soybean yield when controlled at different timings except at highest density planted. A more significant soybean yield loss might have occurred with the higher volunteer corn clump densities.

Results reported in this study indicates that volunteer corn control timings did not have an impact on soybean yield at lower volunteer corn densities ( $\leq 5,000$  plants  $\text{ha}^{-1}$ ) but still an early application of herbicides is recommended from insect resistance management point of view, if volunteer corn plants also express transgenic Bt traits. Volunteer corn plants expressing Bt gene provides extra selection pressure to the targeted

insect pests against Bt toxin (Krupke et al., 2009). Volunteer corn also encourages survival and dispersal of corn rootworm by acting as a host plant and providing feeding options to rootworm larvae in soybean crop; thus, limiting the benefits of corn-soybean rotation (Krupke et al., 2009; Marquardt et al., 2012; Shaw et al., 1978). There is a need to control volunteer corn even if it does not present risk of soybean yield loss in order to reduce the risk of corn rootworms, interference of volunteer corn during harvesting operations, and contamination of harvested soybeans from volunteer corn seeds (Deen et al., 2006). ACCase-inhibitors should be tank mixed with different modes of action herbicides or an integrated volunteer corn management program could be adopted that may include tillage, crop rotation, and improved cultural agronomic practices to maximize control and reduce the potential for evolution of herbicide-resistant weeds.

### **Limitations of Research Project**

Volunteer corn clump density maintained in this project was not more than 500 clumps ha<sup>-1</sup> and most of the late season emergence of volunteer corn was recorded from clumps. Impact of late season volunteer corn on the soybean yield could have been achieved by planting more than 500 ears ha<sup>-1</sup>.

### **Future Directions**

In future, the impact of late season volunteer corn emergence on soybean yield could be studied by maintaining higher number of clumps along with individual plants per hectare.



## Figures



Figure 4.1: Volunteer corn seed and ear planting in the soybean rows.



Figure 4.2: Volunteer corn early emergence as individual and clumps in soybean at 25 d after planting.



Figure 4.3. Late season emergence of volunteer corn as clumps after controlling earlier at R2 soybean stage.

Table 4.1. Effect of glyphosate-resistant volunteer corn densities, control timings, and late-season emergence on glyphosate-resistant soybean yield in field experiments conducted at Clay Center and Lincoln, NE in 2013 and 2014<sup>a</sup>.

Volunteer corn density <sup>c</sup>		Control timing <sup>b,d</sup>	Soybean Yield <sup>e</sup>		
			Clay Center (Irrigated)		Lincoln (Rainfed)
			2013	2014	Combined
—plant ha <sup>-1</sup> —	clumps ha <sup>-1</sup>		kg ha <sup>-1</sup>		
0	0	-	2936 a	5683 a	2416 a
1,250	63	0	3067 a	5621 a	2453 a
1,250	63	V4	2691 a	5453 a	2435 a
1,250	63	V6	2960 a	5474 a	2338 a
1,250	63	R2	2827 a	5617 a	2402 a
2,500	125	0	2789 a	5511 a	2370 a
2,500	125	V4	2929 a	5337 a	2548 a
2,500	125	V6	2815 a	5459 a	2352 a
2,500	125	R2	2936 a	5420 a	2459 a
5,000	250	0	2697 a	5564 a	2356 a
5,000	250	V4	2956 a	5528 a	2322 a
5,000	250	V6	2860 a	5485 a	2402 a
5,000	250	R2	3046 a	5448 a	2558 a
10,000	500	0	2901 a	4994 b	1876 b
10,000	500	V4	2765 a	5417 a	2392 a
10,000	500	V6	2785 a	5265 ab	2431 a
10,000	500	R2	2812 a	5068 b	1968 b
P-value	-	-	0.5044	0.0086	0.0165

<sup>a</sup> Location-by-treatment interaction was significant; therefore, data were presented separately. At Clay Center site, a significant year-by-treatment interaction was observed; therefore, both year data were presented separately. At Lincoln site, year-by-treatment interaction was not significant; therefore, both year data were combined.

<sup>b</sup> Abbreviations: 0, no control; V4, V6, R2, soybean growth stages.

<sup>c</sup> Whole corn ears were planted at 5% of individual kernel density to maintain clumps of volunteer corn in soybean.

<sup>d</sup> Volunteer corn control timings were based on no control and soybean growth stages.

<sup>e</sup> Means within columns with common letter(s) are not significantly different according to Tukey-Kramer's pair-wise comparison test at  $P \leq 0.05$ .

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## **Chapter 5**

### **Herbicide Programs for Control of Glyphosate-Resistant Volunteer Corn in Glufosinate-Resistant Soybean**

#### **Abstract**

Glyphosate-resistant volunteer corn is a significant problem weed in soybean grown in rotation. Soybean growers are looking for alternative herbicides, such as glufosinate, for management of glyphosate-resistant weeds, including volunteer corn. The objectives of this study were to evaluate the efficacy of glufosinate applied at different rates in a single or sequential application; and acetyl-coenzyme A carboxylase- (ACCase) inhibitors applied alone or tank-mixed with glufosinate followed by late-POST glufosinate application for control of glyphosate-resistant volunteer corn in glufosinate-resistant soybean. Field experiments were conducted at Clay Center, NE in 2013 and 2014. Glyphosate-resistant corn was planted at a density of 35,000 seeds ha<sup>-1</sup> to mimic volunteer corn population and glufosinate-resistant soybean was cross planted. Glufosinate applied alone resulted in < 80% control of common waterhemp and volunteer corn at 15 d after early-POST (DAEP) regardless of application rates, while green foxtail control was rate dependent (72 to 93%). The ACCase inhibitors applied alone provided > 93% control of volunteer corn compared to tank-mixed with glufosinate (80 to 82%), except sethoxydim (< 80%) applied alone or in tank-mixed with glufosinate at 15 DAEP. Glufosinate applied at different rates in a single or sequential application usually provided  $\geq$  85% control of common waterhemp, green foxtail, and volunteer corn at 30

DAEP or 15 DALP. The ACCase-inhibitors applied alone or tank-mixed with glufosinate provided  $\geq 97\%$  control of volunteer corn and green foxtail at 15 d after late-POST (DALP) application of glufosinate. At 75 DALP, glufosinate applied at different rates in a single or sequential application resulted in  $\geq 90\%$  control of green foxtail, and volunteer corn and  $> 85\%$  control of common waterhemp. Similar results were reflected for volunteer corn density and biomass at 75 DALP. Green foxtail and volunteer corn usually resulted in zero density and biomass due to higher level of control, while comparatively higher yet similar density and biomass of common waterhemp was reported in all herbicide treatments. Soybean yield was not affected by any of herbicide treatments partially due to hail and wind storm affected plants and pods later in the season in both years.

**Nomenclature:** Clethodim; fenoxaprop; fluazifop; glufosinate; quizalofop; sethoxydim; common waterhemp, *Amaranthus rudis* Sauer; green foxtail, *Setaria viridis* (L.) Beauv; soybean, *Glycine max* (L.) Merr.; volunteer corn, *Zea mays* L.

**Keywords:** Antagonism, herbicide-resistant, weed control.



## Introduction

Over reliance on glyphosate for weed control in corn and soybean in the last 17 yr resulted in the evolution of glyphosate-resistant weeds (Owen, 2008). By 2014, 29 weed species worldwide have evolved resistance to glyphosate, including 14 species in the United States (Heap, 2014). Therefore, alternate herbicide programs are required for control of existing herbicide-resistant weeds and to reduce further evolution of glyphosate-resistant weeds. Before commercialization of glufosinate-resistant corn and soybean, application of glufosinate was limited to non-crop areas, preplant applications, as well as weed control in orchards and vineyards (Coetzer et al., 2002; Singh and Tucker, 1987). However, glufosinate-resistant corn and soybean provided growers an opportunity to apply glufosinate POST for controlling many troublesome weeds.

Glufosinate label recommends effective control of volunteer corn when they are 25- to 30-cm tall (Anonymous, 2014); however, variable control is reported. Shauck and Smeda (2012) reported < 80% control of glyphosate-resistant corn hybrids when glufosinate was applied to 10- and 40-cm tall plants compared to 20-cm (> 80% control) in a corn replant situation. Steckel et al. (2009) reported variability in glufosinate efficacy with height of volunteer corn plants. Glufosinate can be applied sequentially in glufosinate-resistant corn and soybean. Maximum rate of glufosinate per application is 740 g ai ha<sup>-1</sup> with a cumulative 1,340 g ai ha<sup>-1</sup> per growing season (Anonymous, 2014). Earnest et al. (1998) reported ≥ 90% control of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] when glufosinate was applied sequentially in glufosinate-resistant corn. Similarly, Aulakh et al. (2011) reported ≥ 97% control of large crabgrass [*Digitaria*

*sanguinalis* (L.) Scop.], Palmer amaranth, sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby], and smallflower morningglory [*Jacquemontia tamnifolia* (L.) Griseb.] with glufosinate applied in a sequential application. Therefore, sequential application of glufosinate or tank mixing with ACCase-inhibitors may provide better control of glyphosate-resistant volunteer corn in glufosinate-resistant soybean. However, few studies reported that when tank-mixed with ACCase-inhibitors, glufosinate antagonized control of some annual and perennial grasses (Burke et al., 2005; Gardner et al., 2006). A recent survey reported that cultivation of glufosinate-resistant soybean is increasing in the midsouthern United States, specifically for control of glyphosate-resistant Palmer amaranth (Barnett et al., 2013; Aulakh et al., 2013). It is likely that cultivation of glufosinate-resistant soybean may increase in the near future in the Midwest for control of glyphosate-resistant weeds (Kaur et al., 2014) and volunteer corn (Chahal et al., 2014). Scientific literature is not available regarding the efficacy of glufosinate applied alone at different rates or when tank-mixed with ACCase-inhibitors for control of volunteer corn in glufosinate-resistant soybean. Hence, the objectives of this study were to 1) compare efficacy of glufosinate applied at different rates in a single or sequential application for control of glyphosate-resistant volunteer corn, 2) compare efficacy of ACCase-inhibitors applied alone or tank-mixed with glufosinate in an early-POST followed by a late-POST application of glufosinate for control of glyphosate-resistant volunteer corn and other weeds, and 3) evaluate yield of glufosinate-resistant soybean.

## Materials and Methods

Field experiment was conducted at the South Central Agriculture Laboratory (SCAL), University of Nebraska-Lincoln, near Clay Center, NE in 2013 and 2014. The soil texture was silty clay loam with pH of 6.5, 17% sand, 58% silt, 25% clay, and 2.5% organic matter. Glyphosate-resistant volunteer corn scenario was created in the field by planting glyphosate-resistant corn (Cv. 'Mycogen 2G 681') at a density of 35,000 seeds  $\text{ha}^{-1}$  in a 76-cm row spacing on May 23 and May 6 in 2013 and 2014, respectively. Glufosinate-resistant soybean (Cv. 'Stine 30 LC 28') was cross-planted in rows spaced 76-cm apart on May 28 and May 8 in 2013 and 2014, respectively, at a density of 370,500 seeds  $\text{ha}^{-1}$ . The experiment was arranged in a randomized complete block design with four replications. Plots were 3 m wide and 9 m long, comprising four soybean rows.

For the control of grass weeds and early season existing weeds, tank-mixture of S-metolachlor (Dual II Magnum, Syngenta Crop Protection, Inc Greensboro, NC 27419) at 1.63 kg ai  $\text{ha}^{-1}$  and glyphosate (Roundup PowerMAX, Monsanto Company, 800 North Lindberg Ave., St. Louis, Mo) at 1.06 kg ae  $\text{ha}^{-1}$  was applied to the experimental area before 2 d of planting corn. Herbicide treatments included glufosinate applied at different rates in a single or sequential application; ACCase-inhibitors (clethodim, fenoxaprop plus fluazifop, fluazifop, quizalofop, or sethoxydim) applied alone or tank-mixed with glufosinate in an early-POST application and followed by a late-POST application of glufosinate (Table 5.1). A nontreated control was included for comparison. The application rates of herbicides were selected based on recommended labeled rates.

Herbicide treatments were applied with a CO<sub>2</sub>-pressurized backpack sprayer consisting of a four nozzle boom fitted with AIXR 110015 flat-fan nozzles (TeeJet, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189), and was calibrated to deliver 140 L ha<sup>-1</sup> at 276 kPa. Glyphosate-resistant volunteer corn was 25- to 30-cm tall and soybean was at V2 to V3 stage at the time of early-POST application of herbicides (June 26, 2013 and June 10, 2014). Glufosinate at 600 g ai ha<sup>-1</sup> was applied late-POST in selected treatments (Table 5.1) on July 12 and June 26 in 2013 and 2014, respectively when volunteer corn was 32- to 38-cm tall and soybean was at V5 to V6 stage.

Visual control estimates were recorded for volunteer corn and other existing weeds at 15 d after early POST (DAEP) and 15, 30, 45, and 75 d after late POST (DALP) herbicide treatments based on 0 to 100% scale where 0% meaning no control and 100% meaning complete control of volunteer corn and other weeds. The density and biomass of volunteer corn and other weeds were assessed from two randomly selected 0.25 m<sup>2</sup> quadrats per plot at 45 DALP herbicide treatment. Volunteer corn and other weeds were hand harvested separately, oven dried at 65 C, and dry weight was recorded. Soybean was harvested at maturity with a small-plot combine and yields were adjusted to 13% moisture content.

Data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc, Cary, NC). Year-by-treatment interaction was not significant; therefore, treatment was considered as the fixed effect, while year (nested within replication) was considered as random effect in the model. Biomass data of common waterhemp were arc-sine square-root transformed before analysis; however,

data presented are the means of actual values for comparison based on interpretation from the transformed data. Where the ANOVA indicated treatment effects were significant, means were separated at  $P \leq 0.05$  using Tukey-Kramer's pairwise comparison test.

## Results and Discussion

Glufosinate applied at different rates provided variable control (61 to 78%) of volunteer corn at 15 DAEP treatment, while ACCase-inhibiting herbicides applied alone provided  $\geq 93\%$  control, except sethoxydim (76%) (Table 5.2). Similarly, Soltani et al. (2006) reported  $< 80\%$  control of volunteer corn with sethoxydim compared to  $> 85\%$  control with other ACCase-inhibitors at 28 d after treatment. Volunteer corn control was 71 to 82% when ACCase-inhibitors were tank-mixed with glufosinate compared with applied alone at 15 DAEP. This might be due to antagonism which is commonly observed when broadleaf herbicides are tank-mixed with graminicides (Culpepper et al., 1998, 1999; Holshouser and Coble, 1990; Vidrine et al., 1995). For instance, Burke et al. (2005) reported 50% reduction in goosegrass [*Eleusine indica* (L.) Gaertn] control by tank-mixing clethodim with glufosinate compared to clethodim applied alone. No difference in volunteer corn control was observed with sethoxydim applied alone (76%) or tank-mixed with glufosinate (71%). Control of volunteer corn increased (90 to 93%) at 30 DAEP with a single application of glufosinate at  $\geq 600$  g ai ha<sup>-1</sup>; however, glufosinate at 450 g ai ha<sup>-1</sup> resulted in 79% control. Shauck and Smeda (2012) reported 80 to 85% control of 20-cm tall glyphosate-resistant corn with glufosinate at 450 g ai ha<sup>-1</sup>. A followed by late-POST application of glufosinate in a sequential program improved volunteer corn control  $\geq 98\%$  at 15 DALP. Similar level of volunteer corn control ( $\geq 97\%$ ) was observed with ACCase-inhibitors applied alone or tank-mixed with glufosinate when followed by late-POST application of glufosinate. Similarly, Beyers et al. (2002) reported improved control of common waterhemp, giant foxtail, morningglory, and

prickly sida, with a sequential application of glufosinate. At 75 DALP, volunteer corn control was > 90% with all the herbicide treatments. Similar results were reflected in volunteer corn density and biomass. For example, nontreated control had the highest volunteer corn density (17 plants m<sup>-2</sup>) and biomass (230 g m<sup>-2</sup>) followed by single application of glufosinate at 450 g ai ha<sup>-1</sup> (19 g m<sup>-2</sup>) and 600 g ai ha<sup>-1</sup> (13 g m<sup>-2</sup>), while rest of the treatments resulted in no volunteer corn biomass due to the highest level of control (99%) (Table 5.2).

Common waterhemp and green foxtail were the primary weeds (other than volunteer corn) infesting experimental site during both years. Green foxtail emergence was partially due to lack of activation of S-metolachlor because of limited available moisture early in the season during both years. Green foxtail control was affected by glufosinate application rates, providing greater control at 740 g ai ha<sup>-1</sup> (> 90%) followed by 600 (80 to 85%) and 450 g ai ha<sup>-1</sup> (70 to 75%) at 15 DAEP (Table 5.3). Similarly, Bethke et al. (2013) reported greater control (86%) of giant foxtail with glufosinate applied at higher rates compared to the lower rates (73 to 76%). The ACCase-inhibitors applied alone or tank-mixed with glufosinate provided > 90% control of green foxtail, except sethoxydim applied alone (87%) at 15 DAEP. Similarly, Abit et al. (2011) reported > 90% control of green foxtail with quizalofop applied alone. Control of green foxtail was > 90% in all herbicide treatments compared to nontreated control with difference between some treatments at 15 DALP; however, at 75 DALP, all herbicide treatments provided 99% control of green foxtail. Corbett et al. (2004) reported > 95% control of green and yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes] with

single or sequential application of glufosinate at two different rates. Similarly, Johnson et al. (2014) reported > 90% control of johnsongrass by tank-mixing clethodim and glufosinate applied early-POST and followed by a late-POST application of glufosinate. Nontreated control had the highest green foxtail biomass ( $29 \text{ g m}^{-2}$ ), while no biomass was reported and harvested in any of the herbicide treatments (data not shown).

Glufosinate applied in a single application provided  $\leq 77\%$  control of common waterhemp at 15 DAEP with the highest rate provided significantly greater control ( $\geq 76\%$ ) compared with the lower rates ( $< 65\%$ ) (Table 5.4). The ACCase-inhibitors applied alone provided no control of common waterhemp, while their tank-mixed application with glufosinate provided 60 to 65% control. At 15 DALP, 85 to 95% control of common waterhemp was observed with a single application of glufosinate, while glufosinate (irrespective of the rate) sequential application provided  $\geq 97\%$  control. Similarly, Beyers et al. (2002) reported 93% control of common waterhemp with a sequential application compared to a single application of glufosinate (85%). A followed by (sequential) application of glufosinate resulted in > 95% control of common waterhemp compared with < 86% control with a single application. At 75 DALP, all herbicide treatments provided  $\geq 86\%$  control of common waterhemp. Additionally, glufosinate would also be effective for control of glyphosate-resistant common waterhemp, a major problem weed in the Midwest. For instance, Sarangi et al. (2014) reported > 85% control of glyphosate-resistant common waterhemp with a single application of glufosinate at  $594 \text{ g ai ha}^{-1}$ . The highest biomass ( $327 \text{ g m}^{-2}$ ) of common waterhemp was recorded in the nontreated



control plots compared to  $< 70 \text{ g m}^{-2}$  in herbicide treated plots with no difference among them (data not shown).

Year-by-treatment interaction was not significant; therefore, yield data were pooled and combined data are presented. No difference in soybean yield between herbicide treatments was observed, partially due to hail and wind storm affected plants later in the season in both years. Though not statistically different, lower soybean yield was observed in the herbicide treatments included the ACCase-inhibitors applied alone compared to tank-mixed with glufosinate. This might be due to early season competition of common waterhemp with soybean as no control of common waterhemp was achieved until glufosinate was applied late-POST.

Results of this study suggested that glufosinate can effectively control glyphosate-resistant volunteer corn in glufosinate-resistant soybean. The ACCase-inhibitors provided better control of green foxtail and volunteer corn compared to a single application of glufosinate early in the season; however, later in the season, control was comparable. Tank-mixing ACCase-inhibitors with glufosinate applied early-POST reduced efficacy of ACCase-inhibitors for volunteer corn control, but a follow up application of glufosinate provided excellent control of partially controlled volunteer corn. Glufosinate applied in a single or sequential application provided  $> 85\%$  control of glyphosate-resistant volunteer corn along with other weeds; however, herbicide program based on a single herbicide or herbicide with the same mode of action favors the selection pressure and if used repeatedly, results in the evolution of herbicide-resistant weeds. In fact, three weed species have evolved resistance to glufosinate worldwide (Heap, 2014), including Italian

ryegrass, being the only species in the United States (Avila-Garcia et al., 2012).

Therefore, glufosinate should be carefully incorporated in herbicide programs along with herbicides belong to other modes of action in glufosinate-resistant soybean (Johnson et al., 2014).

The primary objective of this study was to control glyphosate-resistant volunteer corn in glufosinate-resistant soybean and PRE herbicides registered in soybean are not effective for control of volunteer corn (Chahal et al., 2014). Therefore, herbicide programs in this study were based on POST herbicides, which are not the best programs for management of other weeds, such as common waterhemp. Several studies reported that use of residual herbicides and herbicides with different modes of action is an important component of weed management program (Aulakh et al., 2012, Whitaker et al., 2011). Therefore, an integrated weed management approach is required for controlling existing herbicide-resistant weeds and to avoid evolution of new herbicide-resistant weeds (Norsworthy et al., 2012).

### **Limitation of Research Project**

Results of this study indicate that a high level of glyphosate-resistant volunteer corn control can be achieved through glufosinate in a single or sequential application; however, glufosinate will not be an effective option under all situations. For instance, glyphosate plus glufosinate resistant corn is available in the marketplace, thus glufosinate will not be an effective option for control of volunteer corn if hybrid corn planted previous year is stacked resistant. Additionally, multiple herbicide resistant crops, including corn resistant to 2,4-D, glyphosate, and glufosinate may commercialize in the

near future (Craigmyle et al., 2013) that will leave ACCase inhibitors as the only option for volunteer corn control.

## Figures

**15 d after early-POST**



**15 d after late-POST**



Figure 5.1. Control of glyphosate-resistant volunteer corn at 15 d after early-and late-POST application of glufosinate.

**Quizalofop alone****Quizalofop tank-mixed with glufosinate**

Figure 5.2. Control of glyphosate-resistant volunteer corn at 15 d after early-POST application of quizalofop alone and quizalofop tank mixed with glufosinate.

## Tables

Table 5.1. Herbicide treatments, application timing, rates, and products used in a field experiment conducted in Nebraska in 2013 and 2014.

Herbicide common name	Timing <sup>a</sup>	Rate g ai ha <sup>-1</sup>	Trade name	Manufacturer	Adjuvant <sup>b</sup>
Glufosinate	E-POST	450	Liberty 280	Bayer Crop Science, Research Triangle Park, NC 27709	AMS
Glufosinate	E-POST	600	Liberty 280	Bayer Crop Science	AMS
Glufosinate	E-POST	740	Liberty 280	Bayer Crop Science	AMS
Glufosinate <i>fb</i>	E-POST	450	Liberty 280	Bayer Crop Science	AMS
glufosinate	L-POST	600	Liberty 280		AMS
Glufosinate <i>fb</i>	E-POST	600	Liberty 280	Bayer Crop Science	AMS
glufosinate	L-POST	600	Liberty 280		AMS
Glufosinate <i>fb</i>	E-POST	740	Liberty 280	Bayer Crop Science	AMS
glufosinate	L-POST	600	Liberty 280		AMS
Clethodim <i>fb</i>	E-POST	140	Select Max	Valent USA Corporation, Walnut Creek, CA 94596	AMS + NIS
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Clethodim + glufosinate <i>fb</i>	E-POST	140 + 600	Select Max + Liberty 280	Valent USA Corporation + Bayer Crop Science	AMS + NIS
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Quizalofop <i>fb</i>	E-POST	40	Assure II	DuPont Crop Protection, P.O.Box 80705 Wilmington, DE 19880	COC
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Quizalofop + glufosinate <i>fb</i>	E-POST	40 + 600	Assure II + Liberty 280	DuPont Crop Protection + Bayer Crop Science	COC
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Fluazifop <i>fb</i>	E-POST	210	Fusilade DX	Syngenta Crop Protection, Inc. Greensboro, NC 27419	NIS + UAN-28
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Fluazifop + glufosinate <i>fb</i>	E-POST	210 + 600	Fusilade DX + Liberty 280	Syngenta Crop Protection + Bayer Crop Science	NIS
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Fenoxaprop + fluazifop <i>fb</i>	E-POST	130	Fusion	Syngenta Crop Protection	COC + AMS
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Fenoxaprop + fluazifop + glufosinate <i>fb</i>	E-POST	130 + 600	Fusion + Liberty 280	Syngenta Crop Protection + Bayer Crop Science	COC + AMS
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Sethoxydim <i>fb</i>	E-POST	350	Poast Plus	BASF Corporation, 26 Davis Drive, Research Triangle Park, NC 27709	COC + AMS
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS
Sethoxydim + glufosinate <i>fb</i>	E-POST	350 + 600	Poast Plus + Liberty 280	BASF Corporation + Bayer Crop Science	COC + AMS
glufosinate	L-POST	600	Liberty 280	Bayer Crop Science	AMS

<sup>a</sup> Abbreviations: E-POST, early POST; L-POST, late POST; AMS, ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA); COC, crop oil concentrate (Agridex, Helena Chemical Co., Collierville, TN); *fb*, followed by; NIS, nonionic surfactant (Induce, Helena Chemical Co., Collierville, TN); UAN-28, Urea ammonia nitrate solution 28% (Sylvite Agri-Services, Ontario, Canada).

<sup>b</sup> AMS at 2% wt/v, COC at 1% v/v, UAN-28 at 2.34 L ha<sup>-1</sup>, and NIS at 0.25% v/v was mixed with herbicides.

Table 5.2. Effect of herbicide treatments on glyphosate-resistant volunteer corn control, density, biomass, and soybean yield in a field experiment conducted in Nebraska in 2013 and 2014<sup>a</sup>.

Herbicide <sup>b</sup>	Timing	Rate <sup>b</sup> g ai ha <sup>-1</sup>	Control			Density <sup>c</sup> no. m <sup>-2</sup>	Biomass <sup>c</sup> g m <sup>-2</sup>
			15 DAEP <sup>b,c</sup>	15 DALP <sup>b,c</sup>	75 DALP <sup>b,c</sup>		
			%				
Nontreated control <sup>d</sup>	-	-	-	-	-	17 a	230 a
Glufosinate	E-POST	450	66 ef	79 c	93 b	3 b	19 b
Glufosinate	E-POST	600	73 de	90 b	96 ab	2 b	13 b
Glufosinate	E-POST	740	75 de	93 b	99 a	0 c	0 c
Glufosinate <i>fb</i>	E-POST	450					
glufosinate	L-POST	600	61 f	98 a	99 a	0 c	0 c
Glufosinate <i>fb</i>	E-POST	600					
glufosinate	L-POST	600	70 ef	98 a	99 a	0 c	0 c
Glufosinate <i>fb</i>	E-POST	740					
glufosinate	L-POST	600	78 de	98 a	99 a	0 c	0 c
Clethodim <i>fb</i>	E-POST	140					
glufosinate	L-POST	600	94 a	99 a	99 a	0 c	0 c
Clethodim + glufosinate <i>fb</i>	E-POST	140 + 600					
glufosinate	L-POST	600	81 bcd	99 a	99 a	0 c	0 c
Quizalofop <i>fb</i>	E-POST	40					
glufosinate	L-POST	600	95 a	98 a	99 a	0 c	0 c
Quizalofop + glufosinate <i>fb</i>	E-POST	40 + 600					
glufosinate	L-POST	600	82 bcd	98 a	99 a	0 c	0 c
Fluazifop <i>fb</i>	E-POST	210					
glufosinate	L-POST	600	96 a	97 a	99 a	0 c	0 c
Fluazifop + glufosinate <i>fb</i>	E-POST	210 + 600					
glufosinate	L-POST	600	80 bcd	97 a	99 a	0 c	0 c
Fenoxaprop + fluazifop <i>fb</i>	E-POST	130					
glufosinate	L-POST	600	93 a	99 a	99 a	0 c	0 c
Fenoxaprop + fluazifop + glufosinate <i>fb</i>	E-POST	130 + 600					
glufosinate	L-POST	600	81 bcd	98 a	99 a	0 c	0 c
Sethoxydim <i>fb</i>	E-POST	350					
glufosinate	L-POST	600	76 cd	99 a	99 a	0 c	0 c
Sethoxydim + glufosinate <i>fb</i>	E-POST	350 + 600					
glufosinate	L-POST	600	71 def	99 a	99 a	0 c	0 c
P-value			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

<sup>a</sup> Year-by-treatment interaction was not significant; therefore, both year data were combined.

<sup>b</sup> Abbreviations: *fb*, followed by; ai, active ingredient; DAEP, days after early-POST; DALP, days after late-POST.

<sup>c</sup> Means within columns with no common letter(s) are significantly different according to Tukey-Kramer's pair-wise comparison test at  $P \leq 0.05$ .

<sup>d</sup> The percent control (0%) data of nontreated control were not included in analysis.

Table 5.3. Effect of herbicide treatments on green foxtail control, density, and biomass in a field experiment conducted in Nebraska in 2013 and 2014<sup>a</sup>.

Herbicide <sup>b</sup>	Timing	Rate <sup>b</sup> g ai ha <sup>-1</sup>	Control		
			15 DAEP <sup>b,c</sup>	15 DALP <sup>b,c</sup>	75 DALP <sup>b,c</sup>
Nontreated control <sup>d</sup>	-	-	%		
Glufosinate	E-POST	450	75 d	91 bc	99 a
Glufosinate	E-POST	600	84 c	90 bc	99 a
Glufosinate	E-POST	740	92 a	94 b	99 a
Glufosinate <i>fb</i>	E-POST	450			
glufosinate	L-POST	600	72 d	93 bc	99 a
Glufosinate <i>fb</i>	E-POST	600			
glufosinate	L-POST	600	81 c	94 bc	99 a
Glufosinate <i>fb</i>	E-POST	740			
glufosinate	L-POST	600	93 a	98 ab	99 a
Clethodim <i>fb</i>	E-POST	140			
glufosinate	L-POST	600	91 a	99 a	99 a
Clethodim + glufosinate <i>fb</i>	E-POST	140 + 600			
glufosinate	L-POST	600	92 a	99 a	99 a
Quizalofop <i>fb</i>	E-POST	40			
glufosinate	L-POST	600	93 a	99 a	99 a
Quizalofop + glufosinate <i>fb</i>	E-POST	40 + 600			
glufosinate	L-POST	600	91 a	99 a	99 a
Fluazifop <i>fb</i>	E-POST	210			
glufosinate	L-POST	600	91 a	99 a	99 a
Fluazifop + glufosinate <i>fb</i>	E-POST	210 + 600			
glufosinate	L-POST	600	90 ab	99 a	99 a
Fenoxaprop + fluazifop <i>fb</i>	E-POST	130			
glufosinate	L-POST	600	91 ab	99 a	99 a
Fenoxaprop + fluazifop + glufosinate <i>fb</i>	E-POST	130 + 600			
glufosinate	L-POST	600	92 a	99 a	99 a
Sethoxydim <i>fb</i>	E-POST	350			
glufosinate	L-POST	600	87 bc	99 a	99 a
Sethoxydim + glufosinate <i>fb</i>	E-POST	350 + 600			
glufosinate	L-POST	600	91 ab	99 a	99 a
P-value			< 0.0001	< 0.0001	< 0.0001

<sup>a</sup> Year-by-treatment interaction was not significant; therefore, both year data were combined.<sup>b</sup> Abbreviations: *fb*, followed by; ai, active ingredient; DAEP, days after early POST; DALP, days after late POST.<sup>c</sup> Means within columns with no common letter(s) are significantly different according to Tukey-Kramer's pair-wise comparison test at  $P \leq 0.05$ .<sup>d</sup> The percent control (0%) data of nontreated control were not included in analysis.



Table 5.4. Effect of herbicide treatments on common waterhemp control, density, and biomass in a field experiment conducted in Nebraska in 2013 and 2014<sup>a</sup>.

Herbicide <sup>b</sup>	Timing	Rate <sup>b</sup> g ai ha <sup>-1</sup>	Control			Density <sup>c</sup> plants m <sup>-2</sup>	Biomass <sup>c,d</sup> g m <sup>-2</sup>	Soybean Yield <sup>c</sup> kg ha <sup>-1</sup>
			15 DAEP <sup>b,c</sup>	15 DALP <sup>b,c</sup>	75 DALP <sup>b,c</sup>			
			%					
Nontreated control <sup>e</sup>	-	-	-	-	-	6 a	327 a	236 b
Glufosinate	E-POST	450	53 d	85 cde	86 a	3 b	67 b	1,960 a
Glufosinate	E-POST	600	61 bcd	93 a-d	88 a	2 b	65 b	1,815 a
Glufosinate	E-POST	740	76 a	94 abc	91 a	1 b	30 b	1,991 a
Glufosinate <i>fb</i>	E-POST	450	56 cd	98 a	92 a	1 b	27 b	1,767 a
glufosinate	L-POST	600						
Glufosinate <i>fb</i>	E-POST	600	63 bcd	97 a	94 a	1 b	19 b	1,859 a
glufosinate	L-POST	600						
Glufosinate <i>fb</i>	E-POST	740	77 a	99 a	94 a	1 b	15 b	1,842 a
glufosinate	L-POST	600						
Clethodim <i>fb</i>	E-POST	140	0 e	86 b-e	93 a	2 b	28 b	1,833 a
glufosinate	L-POST	600						
Clethodim + glufosinate <i>fb</i>	E-POST	140 + 600	62 bcd	95 ab	94 a	1 b	26 b	1,998 a
glufosinate	L-POST	600						
Quizalofop <i>fb</i>	E-POST	40	0 e	81 de	90 a	2 b	34 b	1,672 a
glufosinate	L-POST	600						
Quizalofop + glufosinate <i>fb</i>	E-POST	40 + 600	64 bc	99 a	96a	0.5 b	17 b	1,842 a
glufosinate	L-POST	600						
Fluazifop <i>fb</i>	E-POST	210	0 e	79 e	93 a	1 b	24 b	1,614 a
glufosinate	L-POST	600						
Fluazifop + glufosinate <i>fb</i>	E-POST	210 + 600	66 bc	99 a	96 a	2 b	18 b	1,936 a
glufosinate	L-POST	600						
Fenoxaprop + fluazifop <i>fb</i>	E-POST	130	0 e	82 cde	91 a	2 b	31 b	1,701 a
glufosinate	L-POST	600						
Fenoxaprop + fluazifop + glufosinate <i>fb</i>	E-POST	130 + 600	65 bc	96 ab	96 a	1 b	19 b	1,969 a
glufosinate	L-POST	600						
Sethoxydim <i>fb</i>	E-POST	350	0 e	83 cde	90 a	2 b	37 b	1,795 a
glufosinate	L-POST	600						
Sethoxydim + glufosinate <i>fb</i>	E-POST	350 + 600	63 ccd	95 ab	95 a	1 b	10 b	1,988 a
glufosinate	L-POST	600						
P-value			< 0.0001	< 0.0001	< 0.0031	< 0.0001	< 0.0001	0.7896

<sup>a</sup> Year-by-treatment interaction was not significant; therefore, both year data were combined.

<sup>b</sup> Abbreviations: *fb*, followed by; ai, active ingredient; DAEP, days after early POST; DALP, days after late POST.

<sup>c</sup> Means within columns with no common letter(s) are significantly different according to Tukey-Kramer's pair-wise comparison test at  $P \leq 0.05$ .

<sup>d</sup> Biomass data were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

<sup>e</sup> The percent control (0%) data of nontreated control were not included in analysis.

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